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- (54) Identification method and specific detection method of slow growing mycobacteria utilizing DNA gyrase gene
- (57) A method for identification and detection of slow growing mycobacteria, especially tubercle bacilli group bacteria, utilizing characteristic nucleotide sequences which are present in the *gyrB* gene. It renders possible accurate identification and detection of slow growing mycobacteria which are difficult by the conventional methods.

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to a method for identification or detection of slow growing mycobacteria having a large number of clinical cases as causative microorganisms of tuberculosis and atypical mycobacterial disease (especially, tubercle bacilli group bacteria), which utilizing a nucleotide sequence of a DNA coding for DNA gyrase β subunit (to be referred to as "gyrB" gene" or "gyrB" hereinafter). The identification and detection methods of the present invention are useful in various industrial fields, such as medical science, immunology and veterinary science.

BACKGROUND ART

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[0002] A plurality of species belonging to slow growing mycobacteria are known as acid-fast bacterial species which cause tuberculosis and diseases analogous to tuberculosis in human. Among all, *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex and *Mycobacterium kansasii* occupy the most part of clinical cases. Recently, these bacteria are causing a serious problem for the prognosis of patients of acquired immunodeficiency syndrome (AIDS), because they induce systemic disseminated infection in AIDS patients.

[0003] Conventionally, identification and detection of these bacterial species have been carried out by physiological and biochemical methods based on the culturing. For example, identification and detection have been carried out using such differences in color development because (1) since there are three groups in the slow growing mycobacteria, namely a group which develops yellow color only when it is cultured in the dark after irradiation with light (photochromogen), (2) a group which develops color even when cultured without irradiation of light (scotochromogen) and (3) a group which does not develop color even when light is irradiated (achromogen). Known methods include identification and detection based on the ability of cultured bacteria to produce catalase or show urease activity, Tween hydrolyzing activity or nitrate reducing activity.

[0004] Tuberculosis is a most important infectious disease, because ninety million people in the world newly contract the disease every year and thirty million people of them die every year, but its countermeasure is not sufficient. Tubercle bacilli as its causative agents are classified into four strains, namely a tubercle strain (*Mycobacterium tuberculosis*), a bovine type strain (*Mycobacterium bovis*) and an Africa strain (*Mycobacterium africanum*) which are pathogenic for human and a rat type strain (*Mycobacterium microti*) which is not pathogenic for human. Conventionally, tests of acid-fast bacteria including these mycobacteria were mainly carried out by smear staining by the Ziehl-Neelsen method, isolation culturing method using Ogawa medium and a drug-sensitivity test. With the development of techniques thereafter, BACTEC 460 TB System, Septi-Check AFB, MGIT (Mycobacteria Growth Indicator Tube) and the like novel culture techniques have been developed.

[0005] However, these tests require pure culture. In addition, because phenotype to be compared is liable to change, the judgment often becomes subjective. As a result, not only a prolonged period of time is required but also accurate judgment of the species is extremely difficult. In order to solve such problems, certain identification and detection methods have recently been considered and put into use, e.g., a method for judging the presence of a specific nucleotide sequence of a gene utilizing the polymerase chain reaction (to be referred to as "PCR" hereinafter) or the like, sub-classification of mycobacteria using an insertion sequence IS6110, and the like. The PCR method is suited for identifying and detecting slow growing mycobacteria from the viewpoint that quick and objective judgment is possible without requiring culturing.

In that case, the gene to be used is a rRNA gene in most cases. T. Rogall et al. (1990, J. Gen. Microbiol., 136, 1915 - 1920) have proposed a method for the identification of mycobacteria species based on PCR using 16S 45 rRNA sequences. However, these primers could not distinguish between Mycobacterium gastri and Mycobacterium kansasii, which show different phenotype characteristics. On the other hand, B. Boddinghaus et al. (1990, J. Clin. Microbiol., 28: 1751 - 1759) have reported an oligonucleotide derived from 16S rRNA sequence, which is specific for human type mycobacterium group, avian type mycobacterium-paramycobacterium and Mycobacterium intracellulare group. Even the use of this oligonucleotide could not give necessary resolution for carrying out identification at species level. An identification method using these rRNA gene sequences is now on the market and available from Nippon Roche as a gene diagnosis kit under a trade name of "Amplicore Mycobacterium". In addition to this, detection or identification methods using rRNA sequences have been disclosed by Toyobo (JP-A-10-323189; the term "JP-A" as used herein means an "unexamined published Japanese patent application") and Becton, Dickinson and CO, (JP-A-10-057098). In order to solve the aforementioned problem of not being able to distinguish two species, an identification or detection method using the sequence of a region between 16S rRNA and 23S rRNA has been proposed by A. Roth et al. (1998, J. Clin. Microbiol., 36: 139 - 147). However, since the region between 16S rRNA and 23S rRNA has only about 200 base pairs, it is difficult to carry out high accuracy molecular phylogenic analysis by such a short sequence, and, when a new strain having an intermediate sequence which does not coincide with any one of the sequences of two

strains is generated, it is not able to judge its closeness to which of them.

On the other hand, it was shown that more minute and accurate classification and identification of many bacteria including those of the genus Pseudomonas and the genus Acinetobacter is possible by using a gene which encodes a protein having high evolution rate, particularly a 1,200 bp sequence of gyrB gene (Yamamoto, S. and S. Harayama, 1995, Appl. Environ. Microbiol., 61: 1104 - 1109, Yamamoto, S. and S. Harayama, 1996, Int. J. Syst. Bacteriol., 46: 506 - 511, Harayama, S. and S. Yamamoto, 1996, pp. 250 - 258 in Molecular Biology of Pseudomonas, T. Nakazawa, K. Fukuda, D. Haas, S. Silver (eds), ASM Press, Washington, D.C., S. Yamamoto and S. Harayama, Kagaku-to-Seibutsu (Chemistry and Biology, Japan), 1996, vol. 34, no. 3, pp. 149 - 151, S. Yamamoto and S. Harayama, Nippon Nogei Kagaku Kaishi (Journal of Agricultural Chemistry, Japan), 1997, vol. 71, no. 9, pp. 894 - 897). Attempts have been made to carry out identification of slow growing mycobacteria using genes coding for proteins other than the gyrB gene. For example, C.T. Shivannvar et al. have discussed on the phylogenic relationship among slow growing mycobacteria and their relationship to antigenicity using superoxide dismutase gene (1994, J. Clin. Microbiol., 32: 2801 - 2812), and D.S. Swanson et al. have attempted to carry out minute classification of avian type mycobacterium-paramycobacterium and Mycobacterium Intracellulare group using a 65 kD heat shock protein gene (1997, Int. J. Syst. Bacteriol., 47: 414 - 419). In addition to the rRNA gene, Abbott Laboratories, USA, has disclosed a detection method which uses a gene coding for a protein antigen B of Mycobacterium tuberculosis, gene sequences of 65 kD heat shock protein, 10-kD heat shock proteins and the like of Mycobacterium tuberculosis and sequences related to insertion sequences IS987 and IS6110, in JP-W-10-500567 (the term "JP-W" as used herein means an "Japanese national publication of a PCT application") (International Publication No. WO 95/31571). In addition, Becton, Dickinson and Co. has disclosed in JP-A-06-319560 a detection or identification probe derived from a gene which encodes a 70 kD heat shock protein of Mycobacterium paratuberculosis. However, among these genes, only the gyrB gene shows no contradiction when molecular phylogenical data are compared with the identification of species by the conventional taxonomic means (Yamamoto and Harayama, 1998, Int. J. Syst. Bacteriol., 48: 813 - 819, Yamamoto et al., 199, Int. J. Syst. Bacteriol., 49: 87 - 95, Suzuki et al., Int. J. Syst. Bacteriol., in press, Kasai et al., Int. J. Syst. Bacteriol., in press). A patent application relating to a method for the identification or detection of bacteria using the gyrB gene has already been filed by the present applicant (JP-A-11-16917). However, this document does not disclose identification and detection of slow growing mycobacteria and also does not teach or suggest which region of the gyrB gene can be used in carrying out identification and detection of slow growing mycobacteria.

[0010] Because the slow growing mycobacteria include the bacteria that cause tuberculosis and the like serious diseases, great concern has been directed toward the development of a method for accurately identifying and detecting this bacterial group. On the other hand, because the growth rate of slow growing mycobacteria is lower than that of general bacteria, it is difficult to identify or detect them by physiological and blochemical methods which essentially require culturing of bacteria.

[0011] The present invention has been accomplished under such technical background to provide a method for the identification or detection of slow growing mycobacteria, especially tubercle bacilli group bacteria, utilizing the *gyrB* gene.

[0012] With the aim of solving the aforementioned problems, the present inventors have conducted extensive studies and, as a result, found that at least a part of the nucleotide sequence of *gyrB* DNA is different among the slow growing mycobacteria.

[0013] The present inventors further determined *gyrB* gene sequences of standard strains of the slow growing mycobacteria. Taxonomic positioning of strains isolated from clinical cases was carried out based on these sequences. Then, the resulting taxonomic positioning was checked by the DNA-DNA hybridization method, which is a standard method for identifying species of bacteria. As a result, it was unexpectedly found that the taxonomic positioning determined by using *gyrB* gene sequences shows good agreement with the result of the conventional classification method.

[0014] In addition, nucleotide sequences of *gyrB* fragments were determined by the PCR method by amplifying them from DNA samples of standard strains of atypical mycobacteria, *Mycobacterium gastri* and *Mycobacterium kansasii*, which cannot be distinguished by the nucleotide sequence of 16S rRNA gene which is the most generally used gene sequence-aided detection method of bacteria. When the resulting sequences were compared, it was found that the 16S rRNA gene sequence was identical in both strains, but 66 positions in the 1,257 base *gyrB* gene nucleotide sequence were different in both strains (Figs. 1-11). The present inventors further found that the taxonomically near bacteria belonging to the slow growing mycobacteria can be distinguished by designing primers based on such difference in their sequences, which renders possible the PCR amplification specific for each of these strains. Thus, the present inventors found that it can determine accurate molecular phylogenic position of even a newly isolated strain and also can distinguish related species which cannot be distinguished by other genes, so that it is a method superior to methods by other genes.

[0015] The present invention has been accomplished based on the above knowledge.

SUMMARY OF THE INVENTION

[0016] Thus, the present invention relates to a method for identifying slow growing mycobacteria, especially tubercle bacilli group bacteria, which comprises carrying out identification of bacteria using *gyrB* DNA as a marker. Also, the present invention relates to a method for detecting slow growing mycobacteria, especially tubercle bacilli group bacteria, which comprises carrying out detection of bacteria using *gyrB* DNA as a marker.

[0017] The present invention further relates to a method for identifying slow growing mycobacteria, which comprises amplifying the regions corresponding to SEQUENCE NO. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 in the *gyrB* of slow growing mycobacteria, comparing nucleotide sequences of the amplified fragments with the nucleotide sequences described in SEQUENCE NO. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, thereby calculating genetic distance from each sequence, and carrying out identification of the aforementioned slow growing mycobacteria based on the genetic distance.

[0018] Also the present invention relates to a method for detecting a specific bacterium belonging to the slow growing mycobacteria using a specific sequence in the *gyrB*. In particular, the present invention relates to a method for detecting *Mycobacterium kansasii*, which comprises detecting *Mycobacterium kansasii* using, as a primer or probe, an oligonucleotide that contains a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 4, or its complementary sequence, and also substantially functions as a primer or probe, and to a *Mycobacterium kansasii* detection kit which comprises the just described oligonucleotide.

[0019] The present invention also relates to a method for detecting *Mycobacterium gastri*, which comprises detecting *Mycobacterium gastri* using, as a primer or probe, an oligonucleotide that contains a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 6, or its complementary sequence, and also substantially functions as a primer or probe, and to a *Mycobacterium gastri* detection kit which comprises the just described oligonucleotide.

25 BRIEF DESCRIPTION OF THE DRAWINGS

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Figs. 1 through 11 show alignment of the nucleotide sequences of various slow growing mycobacteria. The symbols at the left side in the figure indicate the organisms shown below.

	KPM1403	Mycobacterium simiae
	KPM1201	Mycobacterium marinum
	KPM2201	Mycobacterium gordonae
35	ATCC25274	Mycobacterium asiaticum
	KPM2027	Mycobacterium scrofulaceum
	KPM2403	Mycobacterium szulgai
	KPM3012	Mycobacterium avium
	Bovine10	Mycobacterium paratuberculo
40	KPM3101	Mycobacterium intracellular
	KPM3401	Mycobacterium malmoense
	ATCC51789	Mycobacterium branderi
	T801	Mycobacterium africanum
	T901	Mycobacterium microtl
45	T704	Mycobacterium bovis
	T021	Mycobacterium tuberculosis
	KPM3504	Mycobacterium gastri
	KPM1001	Mycobacterium kansasii

Fig. 12 shows a result of the identification using the primers based on SEQUENCE No. 1, SEQUENCE NO. 3, and SEQUENCE No. 5. Panel A shows amplified results using *Mycobacterium kansasil*-specific primers (SEQUENCE NO. 1 and SEQUENCE NO. 3), and panel B using *Mycobacterium gastri*-specific primers (SEQUENCE NO. 1 and SEQUENCE NO. 5). Lanes 1 and 12 are molecular weight markers. Lanes 2: strain KPM 1001T, 3: strain KPM 1004, 4: strain KPM 1007, 5: strain KPM KY256, 6: strain KPM KY761, 7: strain KPM KY798, 8: strain KPM 1998-1, 9: strain KPM 3504T, 10: strain KPM 3502 and 11: strain KPM 3503.

Fig. 13 shows a phylogenetic tree of slow glowing mycobacteria prepared by the molecular phylogenic analysis. This figure shows an example in which the presence of new species of slow growing mycobacteria was shown by gyrB sequence analysis. By carrying out molecular phylogenetic analysis and comparing the thus obtained gyrB

sequences with already known gyrB sequences, it was shown that a group of strains KPM 2212, 2014, 1988-5, 2209 and 2013 are new species.

Fig. 14 is an electrophoresis photograph of products amplified by PCR using primers specific for bacteria which constitute the tubercle bacilil.

Fig. 15 is an electrophoresis photograph of products amplified by PCR using primers specific for each bacterium which constitutes the tubercle bacilli.

Fig. 16 is an electrophoresis photograph of fragments prepared by digesting PCR products with restriction enzymes.

10 DETAILED DESCRIPTION OF THE INVENTION

[0021] The following describes the present invention in detail.

[0022] As the first step for the detection or identification of the slow growing mycobacteria (especially tubercle bacilli group bacteria), a sample for detection or identification is collected. Examples of the sample include a sample collected from organisms (human, animals, etc.) showing tuberculosis or tuberculosis-analogous symptoms as well as a strain isolated from the sample. Examples of tuberculosis or tuberculosis-analogous symptoms include pneumonla, empyema, cystitis, pyelonephritis, prostatitis, peritonitis, pericarditis, meningitis, encephalitis, etc. (Pocket guide to clinical microbiology 2nd edition, Oatrick R. Murray, ASM press). The collected sample may be cultured or the microorganism in the sample may be isolated and cultured for the use in the following steps. However, the present invention is advantageous in that the sample as it is can be used.

[0023] Then, a sample or isolated microorganism is usually subjected to a treatment to destroy the cell to extract DNA from the cell. The method for this treatment is not particularly limited and includes physically destroying method, chemically destroying method, etc.

Examples of the method for determining the DNA sequence include the dideoxy terminator method (Molecular Cloning: a laboratory manual 2nd edition, J. Sambrook, E. F. Rritsch, T. Maniatis, CSH press). The sequence determined is compared with the sequences of the DNA gyrase β subunit of the slow growing mycobacteria (Sequence No. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39) to determine whether the microorganism in the sample belongs to the one of these slow growing mycobacteria or is a new species. For the determination, 85% to 100% homology with respect to the 1200 bp sequence of the DNA gyrase β subunit means the same species, while the homology less than 85% means a new species. As a surprising matter, the identification of the slow glowing mycobacteria based on the DNA gyrase β subunit well matches with the identification of the slow glowing mycobacteria by the conventional method. Thus, the present invention makes it possible to identify the slow growing mycobacterial in an accurate way and also makes it possible to distinguish the related species, which are not possible by the conventional way.

Without determining the whole sequence of the DNA gyrase β subunit, identification and detection of the slow growing mycobacteria according to the present invention can be carried out by utilizing one or more unique partial sequence in the DNA gyrase β subunit which is characteristic to one or more of the microorganisms belonging to the slow growing mycobacteria or related species thereof. Example of the unique sequence is a sequence having 0 or more, preferably at least 1, more preferably at least 2, and most preferably at least 3 unique bases in the sequence having a length of 5-mer to 50-mer, preferably 10-mer to 40-mer, more preferably 15-mer to 30-mer. When the unique sequence does not have a unique base, at least one unique base should exist at the 3'-side or 5'-side nearest neighbor base to the unique sequence. The complementary sequence to the unique sequence can be also used.

The unique base means a base which can be found in only one or only several related species among the slow growing mycobacteria. The unique base may be located at arbitrary position in the unique sequence. When the unique sequence is utilized as a primer for the PCR, a unique base located near the 3'-end is preferable for the 5'-end primer and a unique base located near the 5'-end is preferable for the 3'-end primer. For the method utilizing the gel electrophoresis described below, the unique sequence may be designed so that the 3'-side or 5'-side nearest neighbor base to the unique sequence is the unique base (i.e., the unique base is not contained in the unique sequence). Even if a unique sequence which is unique to a certain one species among the slow growing mycobacteria is not found, identification or detection of the certain species is possible by using in combination two or more unique sequences which are respectively unique to several species among the slow growing mycobacteria. For example, four tubercle bacilli group bacteria can be identified or detected by using Sequence 41 shown in Fig. 1. By using Sequence 55 in combination of Sequence 41, it is possible to identify or detect Mycobacterium microtl. Mycobacterium kansasii and Mycobacterium gastri can be identified or detected by using Sequence 1 shown in Fig. 1. By using Sequence 3 in combination of Sequence 1, it is possible to identify or detect Mycobacterium kansasii. According to the present invention, a sample obtained from human or animals showing tuberculosis or tuberculosis-analogous symptoms is used. Accordingly, it is possible to avoid pseudo positive reaction even if there are microorganisms other than slow growing mycobacteria that have the same unique sequence in the DNA gyrase $\boldsymbol{\beta}$ subunit.

Examples of the concrete methods for identifying or detecting the slow growing mycobacteria, which utilizes the unique sequence, a partial sequence in the unique sequence, or a sequence having a unique sequence, in the DNA gyrase β subunit include (1) DNA chip (DNA microarray) (Gingeras et al., 1998, Genome Res. 8: 435-448; Troesch et al. 1999 J. Clin. Microbiol. 37: 49-55), (2) PCR using the same as primers (Kasai, H., Ezaki, T., Harayama, S. 2000. J. Clin. Microbiol. 38: 301-308), (3) hybridization using the same as a probe (de los Reyes et al. 1997. Appl. Environ. Microbiol. 63: 11007-1117), (4) cleavage by the restriction enzyme that recognizes the unique sequence (Kasai H., Ezaki, T., Harayama, S. 2000. J. Clin. Microbiol. 38: 301-308), and the like. Examples of the method to confirm the result of these methods include a method to confirm the existence of the amplified or cleaved fragments by the gel electrophoresis, a method using DNA chip (DNA microarray), etc. The above-described methods can be carried out by the known way (cf. Molecular Cloning: a laboratory manual 2nd edition, J. Sambrook, E. F. Fritssh, T. Maniatis, CSH press; Current protocols of molecular biology edited by Ausubel et al. Wiley; PCR primer - A laboratory manual. edited by Diffenbach & Dveksler. SCH press, all herein incorporated by reference) The identification method and the detection method according to the present invention are further described below.

(1) Identification method

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[0028] The method of the present invention for identifying slow growing mycobacteria is characterized in that the regions corresponding to SEQUENCE NOS. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 in the *gyrB* of slow growing mycobacteria are amplified by PCR, nucleotide sequences of the amplified fragments are compared with the nucleotide sequences described in SEQUENCE NOS. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, thereby calculating genetic distance from each sequence, and then identification of the aforementioned slow growing mycobacteria is carried out based on the genetic distance.

[0029] The term "identification" as used herein means that taxonomic positions of bacteria are determined by a molecular phylogenic or the like means.

[0030] Though not particularly limited, the primers represented by SEQUENCE NO. 59 and SEQUENCE NO. 60 can be exemplified as the primers to be used in the amplification of the regions corresponding to SEQUENCE NOS. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 in the *gyrB*.

[0031] Relationship between the nucleotide sequences of SEQUENCE NOS. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 and their corresponding amino acid sequences and names of original microorganisms is shown in the following table.

TARLE 1

	IABLE 1	
Nucleotide sequence	Amino acid sequence	Name of original microorganisms
SEQUENCE NO. 7	SEQUENCE NO. 8	Mycobacterium simiae
SEQUENCE NO. 9	SEQUENCE NO. 10	Mycobacterium bovis
SEQUENCE NO. 11	SEQUENCE NO. 12	Mycobacterium szulgai
SEQUENCE NO. 13	SEQUENCE NO. 14	Mycobacterium malmoense
SEQUENCE NO. 15	SEQUENCE No. 16	Mycobacterium intracellulare
SEQUENCE NO. 17	SEQUENCE NO. 18	Mycobacterium avium
SEQUENCE NO. 19	SEQUENCE NO. 20	Mycobacterium gordonae
SEQUENCE NO. 21	SEQUENCE NO. 22	Mycobacterium africanum
SEQUENCE NO. 23	SEQUENCE NO. 24	Mycobacterium tuberculosis
SEQUENCE NO. 25	SEQUENCE NO. 26	Mycobacterium gastri
SEQUENCE NO. 27	SEQUENCE NO. 28	Mycobacterium marinum
SEQUENCE NO. 29	SEQUENCE NO. 30	Mycobacterium microti
SEQUENCE NO. 31	SEQUENCE NO. 32	Mycobacterium asiaticum
SEQUENCE NO. 33	SEQUENCE NO. 34	Mycobacterium scrofulaceum
SEQUENCE NO. 35	SEQUENCE NO. 36	Mycobacterium branderi
SEQUENCE NO. 37	SEQUENCE NO. 38	Mycobacterium paratuberculosis

TABLE 1 (continued)

Nucleotide sequence	Amino acid sequence	Name of original microorganisms
SEQUENCE NO. 39	SEQUENCE NO. 40	Mycobacterium kansasii

[0032] The genetic distance can be calculated in accordance, for example, with the method described by Felsenstein in the Phylip program (Felsenstein, J., 1993 PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle, U.S.A.).

(2) Specific detection

[0033] The method of the present invention for detecting *Mycobacterium kansasii* is characterized by the use, as a primer or probe, of an oligonucleotide which contains a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 4, or its complementary sequence, and functions substantially as a primer or probe. Also, the *Mycobacterium kansasii* detection kit of the present invention is characterized in that it contains the just described oligonucleotide.

[0034] The method of the present invention for detecting *Mycobacterium gastri* is characterized by the use, as a primer or probe, of an oligonucleotide which contains a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 6, or its complementary sequence, and also functions substantially as a primer or probe. Also, the *Mycobacterium gastri* detection kit of the present invention is characterized in that it contains the just described oligonucleotide.

[0035] In this connection, the term "substantially functions as a primer or probe" means that the oligonucleotide has such a length that a specific annealing or hybridization can be effected, and its gist is to exclude the oligonucleotide which has a sequence that can anneal to or hybridize with the DNA to be detected but cannot be used in specific detection, because it frequently causes nonspecific annealing or hybridization due to its short length. In order to confirm that a certain oligonucleotide can substantially functions as a primer for PCR, the PCR is carried out at a 3°C higher annealing temperature and a 3°C lower annealing temperature than the usually employed temperature for the PCR. If the PCR product is observed only at 3°C lower annealing temperature, there is a possibility of false-positive. In such a case, the nucleotide sequence of the amplified fragment is determined by the conventional way and compared with the known sequence to confirm whether the oligonucleotide used can substantially work as a primer. In order to confirm that a certain oligonucleotide can substantially function as a primer for PCR, it is preferable to perform PCR by using DNA of already known strain (for example, type strain) as a template for positive and negative controls.

[0036] Though not particularly limited, the oligonucleotide represented by SEQUENCE NO. 3 can be exemplified as an oligonucleotide which can be used in the detection of *Mycobacterium kansasii*, and the oligonucleotide represented by SEQUENCE NO. 5 can be exemplified as an oligonucleotide which can be used in the detection of *Mycobacterium gastri*.

[0037] Preparation of DNA to be tested, preparation of primers and PCR using the same, and preparation of probes and hybridization using the same can be carried out in the usual way without requiring special techniques.

[0038] Regarding the primers to be used in PCR, it is not always necessary that both of them can perform specific annealing, and one of them may perform nonspecific annealing. The primer represented by SEQUENCE NO. 1 can be cited as an example of such a primer which performs nonspecific annealing.

[0039] The methods of the present invention for identifying and detecting the slow growing mycobacteria (especially, tubercle bacilli group bacteria) are characterized in that *gyrB* DNA is used as a marker. Examples of the slow growing mycobacteria include those shown in Table 1. Examples of the tubercle bacilli group bacteria include *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum* and *Mycobacterium microti*.

[0040] The following four methods can be exemplified as the identification and detection methods which use gyrB DNA as a marker.

A) A method which employs PCR

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[0041] This method is carried out as follows.

(1) An oligonucleotide which contains a region of *gyrB* DNA, a region that has different nucleotide sequence among tubercle bacilli group bacteria, is synthesized. Since the nucleotide sequence of *gyrB* DNA corresponding to each bacterium is already determined as shown in Figs. 1-11, the just described oligonucleotide can be synthesized based on these drawings. As the oligonucleotide, an oligonucleotide which encodes the amino acid sequence described in SEQUENCE NO. 46, SEQUENCE NO. 48, SEQUENCE NO. 50, SEQUENCE NO. 52, SEQUENCE NO. 54, SEQUENCE NO. 56 or SEQUENCE NO. 58 can be exemplified as a preferable oligonucleotide, and an oli-

gonucleotide represented by SEQUENCE NO. 45, SEQUENCE NO. 47, SEQUENCE NO. 49, SEQUENCE NO. 51, SEQUENCE NO. 53, SEQUENCE NO. 55 or SEQUENCE NO. 57 can be exemplified as a particularly preferable oligonucleotide.

- (2) A solution which contains the oligonucleotide synthesized in the above step, dNTP, DNA polymerase and a bacterial DNA to be used as a sample is prepared. Concentration of each component contained in the solution may be the same as that in the reaction solution used in general PCR. It is not necessary to purify the bacterial DNA to be used as a sample, and disrupted cells may be used as such for example.
- (3) The solution prepared in the above step is repeatedly heated under such conditions that PCR can be generated. The heating temperature, cycle and the like conditions are not particularly limited, with the proviso that they are within such ranges that PCR can be effected, but, since the homology of *gyrB* DNA among tubercle bacilli group bacteria is high as shown in Figs. 1-11, it is desirable to set the temperature at the time of annealing to a fairly high level. Illustratively, it is desirable to set at 68°C or more. When the synthesized oligonucleotide can be hybridized with the added bacterial DNA, PCR occurs by the repetition of heating and amplified product is formed thereby. On the other hand, when the synthesized oligonucleotide cannot be hybridized with the added bacterial DNA, PCR does not occur and amplified product is not formed.
- (4) Electrophoresis of the solution after the above treatment is carried out. When the amplified product is contained in the solution, its corresponding band is formed on the electrophoresis gel. In consequence, identification and detection of the bacterium of interest can be made based on the electrophoresis pattern.

20 B) A method which uses restriction enzyme digestion fragments

[0042] This method is carried out as follows.

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- (1) An oligonucleotide which is identical to a part of *gyrB* DNA of tubercle bacilli group bacteria and a complementary oligonucleotide of the aforementioned part of DNA are synthesized. Since the nucleotide sequence of *gyrB* DNA corresponding to each bacterium is already determined as shown in Figs. 1-11, the bust described oligonucleotides can be synthesized based on these drawings. As preferred oligonucleotides, an oligonucleotide which encodes the amino acid sequence described in SEQUENCE NO. 42 and an oligonucleotide which encodes the amino acid sequence described in SEQUENCE NO. 44 can be exemplified, and the oligonucleotide represented by SEQUENCE NO. 41 and the oligonucleotide represented by SEQUENCE NO. 43 can be exemplified as particularly preferred oligonucleotide.
- (2) PCR is carried out using the two oligonucleotides synthesized in the above step as primers, and a bacterial DNA sample as a template. It is not necessary to purify the bacterial DNA to be used as a sample, and disrupted cells may be used as such for example. PCR can be carried out in the usual way.
- (3) The DNA fragment amplified in the above step is digested with restriction enzymes. The restriction enzymes to be used are not particularly limited, with the proviso that they can generate different fragments among corresponding bacteria which constitute tubercle bacilli. For example, *Rsa* I and *Taq* I can be exemplified as such restriction enzymes.
- (4) Electrophoresis of the fragments digested in the above step is carried out. The digested DNA fragments appear at positions corresponding to their length. In consequence, identification and detection of the bacterium of interest can be made based on the electrophoresis pattern.

[0043] The aforementioned two methods can be cited as typical examples of the Identification or detection method of the present invention, but other methods are also included in the Identification or detection method of the present invention, with the proviso that they use *gyrB* DNA as a marker. Examples of these other identification or detection methods include a method in which *gyrB* DNA is amplified by PCR, and identification or detection of the bacterium of interest is carried out by determining nucleotide sequence of the amplified fragment and a method in which an oligonucleotide which contains a region of *gyrB* DNA, a region that has different nucleotide sequence among tubercle bacilli group bacteria, is synthesized, and the bacterium of interest is identified or detected by carrying out Southern blotting using the oligonucleotide as a probe.

C) Method which employs the gel electrophoresis

[0044] According to this method, easy and qualitative analysis as well as a certain degree of quantitative analysis are possible for *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. kansasil*, *M. avium*, *M. intracellulare* and for the multiple infection found in the patient having lowered immunological competence.

[0045] Dideoxy nucleotides and primers that are unique to one or more of these species are used for this method. The oligonucleotide used as a primer is designed so that the 3'-side nearest neighbor base in DNA gyrase ß subunit

sequence to the primer is the unique base. The sequence of the primer itself may be common in all of the slow growing mycobacteria or may be common in one or more slow growing mycobacteria. In the latter case, an oligonucleotide mixture is preferably used in order to assure the reaction.

[0046] All of the 4 types of dideoxy nucleotides is labeled with a fluorescent substance or radioactive substance. By labeling each of the 4 dideoxy nucleotides with different types of substances, it is possible to obtain the necessary information from only one lane of the sequence gel. Labeling the 4 dideoxy nucleotides with the same fluorescent substance or radioactive substance requires to carry out electrophoresis using 4 different lanes.

The reaction mixture used for this method is the same with the reaction mixture for the usual sequence reaction except that dATP, dTTP, dGTP, and dCTP are not contained. In other words, the reaction mixture contains, as essential components, an appropriate buffer, a DNA polymerase, a labeled ddNTP, and the primer. A sample collected from the patient is mixed with this reaction mixture and then subjected to the reaction at an appropriate temperature at which the reaction can occur (for example, at 95°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes; 25 cycles). Then, existence of the labeled primer is checked, for example, by subjecting the reaction product to the gel electrophoresis by the conventional way, or the like. The pattern which appears on the gel differs depending on the length of the primer used and the type of the 3'-side nearest neighbor based to the primer. The location of the primer sequence is not particularly limited as long as the length of the primer sequence is the same. However, for the quantitative analysis, primers having a quite high Δ Tm (about 5°C or more) is not preferable. Examples of the primers include the nucleotides represented by Sequence 61 (5'-gacgcstaygcgatatc-3') based on the $\it M.~tuberculosis$ complex and $\it M.~tuberculosis$ kansasii and Sequence 62 (5'-agcggytacaacgtcag) based on M. avium and M. intracellulare in Fig. 1. At the position corresponding to the 18-base length, a signal of "T" is found in the case of M. tuberculosis complex, "G" for M. kansasii, and "C" for M. intracellulare. Detection of a plural number of signals at the position corresponding to the 18-base length means the multiple infection. Moreover, approximate amount of existence of each species can be estimated from the signal intensity detected. The sequence that is unique to 4 species of the tubercle bacilli group bacteria and the sequence which can distinguish M. gastri (a species which is near to M. kansasii but has only a few number of clinical cases of human infection) have been explained in the above-described methods. By combining this method which uses gel electophoresis with the above-described methods, further detailed identification or detection is possible.

D) Method which employs the DNA chip

[0048] The detection or identification of the slow growing mycobacteria is also possible by utilizing the DNA chip. Examples of the method which employs the DNA chip is described below. First, the region in the DNA gyrase β subunit in one or more standard strains of the slow growing mycobacteria is amplified, for example, by the PCR. Then, the amplified product is labeled by Cy5 or the like, and the synthesized DNA oligo probe is fixed on a plate such as slide grass. A DNA in a sample is obtained and subjected to a hybridization reaction on the plate having a solid phrased probe, which is then subjected to washing and detection in the conventional way. The size of the region in the DNA gyrase β subunit is preferably 250 bp or less, more preferably 180 bp or less, and still more preferably 125 bp or less. The oligo probe size is preferably from 14 to 17 mer.

[0049] Known protocols for the method which employs the DNA chip can be employed for the above-described method (cf. Lemieux, B., Aharoni, A., and M. Schena (1998), Overview of DNA Chip Technology, Molecular Breeding, 4, 277-289; Schena, M., Heller, R.A., Theriault, T.P., Konrad, K., Lachenmeier, E., and R.W. Davis (1998), Microarrays: biotechnology's discovery platform and functional genomics, Trends in Biotechnology, 16, 301-306; and Heller, R.A., Schema, M., Chai, A., Shalom, D., Bedilion, T., Glimore, J., Woolley, D.E., and Davis, R.W. (1997), Discovery and analysis of inflammatory disease-related genes using cDNA microarrays, Proceedings of the National Academy of Sciences USA, 94, 2150-2155), all herein incorporated by reference).

45 [0050] Illustrative but non-limiting example of the protocol for the DNA chip method is described below.

(1) Labeling

[0051] PCR amplification product (125 bp) is obtained by 40-cycles treatment (each cycle: at 96°C for 1 minute, 55°C for 30 seconds, and 72°C, 2 minutes). The product is then subjected to the ethanol precipitation. Then, 10 µM Cy5-dCTP/100 µl is added instead of dCTP/100 µl and the 40-cycles treatment was carried out again (each cycle: at 96°C for 1 minute, 55°C for 30 seconds, and 72°C, 2 minutes). Then, the product is subjected to the ethanol precipitation.

55 (2) Spotting

[0052] Spotting is carried out under the following conditions.

Slide: Silylated Slides

Spotting: Spotting by SPBIO (manufactured by HITACHI) using a 4 Pin head with a pitch of 1.0 mm. A 20 μ l sample (10 μ l of 200 μ M probe + 10 μ l of \times 2 Spotting Solution (ArrayltTM)) is used for each well of the plate (about 4 to 5 nl per 1 spot).

Time: about 10 minutes/slide (96 spots)

Oligo probe size: conc. probe 14 - 17 mer, final conc. 100 µM

(3) Hybridization

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10 [0053] Hybridization is carried out using UniHyb™ (Arraylt™). The labeled product is dissolved in 4 μl of sterilized water and 16 μl of ×1.25 UniHyb™ was added.

[0054] Then, 9.6 μ l of the resulting mixture was dropped onto a cover slip (24 × 32 mm; 1.25 μ l/cm²) and the cover slip was placed onto the microarray such that bubbles are not included between the cover slip and the microarray. Then, the microarray is incubated at 46°C for 4 hours.

(4) Washing

[0055] Washing is carried out with $2 \times SSC$ (+ 0.2% SDS) for 5 minutes at room temperature, with 0.1 $\times SSC$ (+ 0.2% SDS) for 5 minutes at room temperature, and then with 0.1 $\times SSC$ (+ 0.2% SDS) for 5 minutes at room temperature. The microarray is centrifuged and dried. At least 1 week storage at 4°C is possible.

(5) Scanning

[0056] Scanning is carried out by using ScanArray 1000 (ScanArray Lite) manufactured by GSI LUMONICS.

Scanning software: ScanArray Analyzing software: QuantArray

EXAMPLE 1

Using the oligonucleotides represented by the nucleotide sequences described in SEQUENCE NO. 39 and SEQUENCE NO. 40, gyrB gene sequences of B acid-fast bacterial strains (KPM 2201T, KPM 2202, KPM 2203, KPM 2013, KPM 2014, KPM 1988-5, KPM 2209 and KPM 2212) isolated from clinical cases were determined. Using the thus obtained gyrB sequences and a gyrB sequence set (SEQUENCE NO. 7 to SEQUENCE NO. 40) for slow growing mycobacteria identification use, their phylogenic relationship was estimated by a molecular phylogenic analysis. The molecular phylogenic analysis was carried out in the following manner using general-purpose molecular phylogenic analysis programs Clustal W (Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994, Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice., Nucleic Acids Res., 22: 4673 - 4680) or Phylip (Felsenstein, J., 1993 PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle, U.S.A.), in accordance with the instructions for use of both programs. A multiple alignment file is prepared by the Clustal W program from the gyrB gene sequences obtained using the oligonucleotides represented by the nucleotide sequences described in SEQUENCE NO. 59 and SEQUENCE NO. 60 and the slow growing mycobacteria identification gyrB sequence set of SEQUENCE NO. 7 to SEQUENCE NO. 40. An example of the parameters to be used in making the multiple alignment is "Gap Open Penalty: 15.00; Gap Extension Penalty: 6.66; DNA weight matrix: IUB; DNA transition weight: 0.5°. The thus obtained multiple alignment is compared with a multiple alignment file obtained from amino acid sequences, and questionable points are corrected. Next, the genetic distance between respective sequences is calculated based on the multiple alignment file. The dnadist program of Phylip is used for the calculation. The calculation is carried out in accordance with the Kimura 2-parameter model. A phylogenetic tree is prepared from the thus obtained 50 genetic distances by a neighboring sequences binding method. Correctness of the phylogenetic tree is checked by calculating the bootstrap probability.

[0058] On the other hand, the aforementioned 8 strains were also identified by a 16S rRNA gene-aided method and a biochemical method. The above results are shown in Table 2.

TABLE 2

Strain name	Biological test	16S rRNA gene	DNA homology test
KPM 2201T	M. gordonae	M. gordonae	M. gordonae
KPM 2202	M. gastri	M. gordonae	M. gordonae
KPM 2203	M. gastri	M. gordonae	M. gordonae
KPM 2013	M. scrofulaceum	M. gordonae	new species
KPM 2014	M. scrofulaceum	M. gordonae	new species
KPM 1988-5	M. scrofulaceum	M. gordonae	new species
KPM 2209	M. scrofulaceum	M. gordonae	new species
KPM 2212	no data	M. gordonae	new species

[0059] As shown in the table, 3 of the above 8 strains, namely KPM 2201T, KPM 2202 and KPM 2203, were identified as strains belonging to *Mycobacterium gordonae*, but the other 5 strains, KPM 2013, KPM 2014, KPM 1988-5, KPM 2209 and KPM 2212, were suggested to be sibling species of *Mycobacterium gordonae* but different species (new species) (Fig. 13). When a DNA-DNA hybridization test (Ezaki, T., Hashimoto, Y., Takeuchi, T., Yamamoto, H, Shu-Lin Liu, Matsui, K. & Yabuuchi, E (1988), *J. Clin. Microbiol.*, 26, 1708 - 1713; Ezaki, T., Hashimoto, Y., Takeuchi & Yabuuchi, E (1989), *Int. J. Syst. Bacteriol.*, 39, 224 - 229) was carried out in order to inspect this result, it was supported that they are new species. This result suggests that the *gyrB* sequence set for slow growing mycobacteria identification use gives highly reliable results for not only known strains but also strains of new species.

EXAMPLE 2

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[0060] Nucleotide sequences of the gyrB gene of Mycobacterium kansasil and Mycobacterium gastri (Figs. 1-11) were compared to prepare a primer which specifically anneals to the gyrB gene of Mycobacterium kansasil (SEQUENCE NO. 3) and a primer which specifically anneals to that of Mycobacterium gastri (SEQUENCE NO. 5). A primer which anneals to the gyrB gene of both strains (SEQUENCE NO. 1) was also prepared.

[0061] Using these primers, PCR was carried out on disrupted cell suspensions of strains KPM 1004, KPM 1007, KPM KY256, KPM KY761, KPM KY768, KPM 1998-1, KPM 3502 and KPM 3503 isolated from clinical cases.

[0062] The PCR amplification conditions are as follows.

At 95°C for 10 minutes; 1 cycle
At 95°C for 1 minute and 68°C for 1 minute and 30 seconds; 30 cycles
At 72°C for 10 minutes; 1 cycle

Primer concentration; 1 µM for each

dNTP: 100 μM for each

Ampli Taq GOLD™ and PCR buffer I attached thereto (Perkin Elmer, USA) were used.

[0063] When the thus amplified DNA fragments were analyzed by an electrophoresis, amplified fragments were observed only by the combination of SEQUENCE NO. 1 and SEQUENCE NO. 3 in the case of KPM 1004, KPM 1007, KPM KY256, KPM KY761, KPM KY768 and KPM 1998-1 (Table 3), so that these strains were identified as *Mycobacterium kansasii*. In the case of KPM 3502 and KPM 3503, amplified fragments were observed only by the combination of SEQUENCE NO. 1 and SEQUENCE NO. 5 (Table 3), so that these strains were identified as *Mycobacterium gastri*. The electrophoresis patterns used in the judgment are as shown in Fig. 12. These identification results coincided with the identification results of the DNA-DNA hybridization method.

TABLE 3

	M. kansasii	M. gastri
SEQUENCE NO. 1	amplification was possible	No amplification
SEQUENCE NO. 3		•

TABLE 3 (continued)

	M. kansasii	M. gastri
SEQUENCE NO. 1	No amplification	amplification was possible
SEQUENCE NO. 5		

EXAMPLE 3

10 [0064] A 10 ng portion of purified DNA was prepared from each of 9 bacterial species including 4 tubercle bacilil group bacterial species and 5 other bacterial species belonging the genus Mycobacterium. PCR was carried out using these DNA samples as templates, and the oligonucleotides described in SEQUENCE NO. 41 and SEQUENCE NO. 43 as primers. The PCR amplification conditions are as follows.

15 At 95°C for 10 minutes; 1 cycle

At 95°C for 1 minute, 68°C for 1 minute and 72°C for 1 minute; 30 cycles

At 72°C for 10 minutes; 1 cycle Primer concentration; 1 µM for each

dNTP: 100 μM for each

Ampli Tag GOLD™ and PCR buffer 1 attached thereto (Perkin Elmer, USA) were used.

[0065] The products amplified by PCR were analyzed by an agarose gel electrophoresis. The results are shown in Fig. 14. In this connection, the relationship between lanes and bacterial species is as follows.

25 Lane 1: Mycobacterium tuberculosis

Lane 2: Mycobacterium bovis

Lane 3: Mycobacterium africanum

Lane 4: Mycobacterium microti

Lane 5: Mycobacterium kansasii

Lane 6: Mycobacterium gastri

Lane 7: Mycobacterium abscessus
Lane 8: Mycobacterium chelonae

Lane 8: Mycobacterium chelonae Lane 9: Mycobacterium trviale

35 EXAMPLE 4

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[0066] A 10 ng portion of purified DNA was prepared from each of 4 tubercle bacilli group bacterial species. PCR was carried out using these DNA samples as templates, and the oligonucleotides represented by SEQUENCE NO. 45, SEQUENCE NO. 47, SEQUENCE NO. 49, SEQUENCE NO. 51, SEQUENCE NO. 53, SEQUENCE NO. 55 and SEQUENCE NO. 57 as primers. The PCR amplification conditions are as described in Example 3. The products amplified by PCR were analyzed by an agarose gel electrophoresis. The results are shown in Fig. 15. In this case, the relationship between lanes and bacterial species is as follows.

Lane 1: Mycobacterium tuberculosis

45 Lane 2: Mycobacterium bovis

Lane 3: Mycobacterium africanum

Lane 4: Mycobacterium microti

[0067] As shown in Fig. 15, the amplified product was observed only by *Mycobacterium tuberculosis* when the oligonucleotides represented by SEQUENCE NO. 45 and SEQUENCE NO. 47 were used as primers, the amplified product was observed only by *Mycobacterium bovis* when the oligonucleotides represented by SEQUENCE NO. 49 and SEQUENCE NO. 51 were used as primers, the amplified product was observed by *Mycobacterium africanum* and *Mycobacterium microti* when the oligonucleotides represented by SEQUENCE NO. 45 and SEQUENCE NO. 53 were used as primers and the amplified product was observed by only *Mycobacterium microti* when the oligonucleotides represented by SEQUENCE NO. 55 and SEQUENCE NO. 57 were used as primers. Based on the above results, relationship between primers and bacterial species can be summarized as follows.

TABLE 4

5	SEQUENCE No.	Mycobacterium tuber- culosis	Mycobacterium bovis	Mycobacterium afri- canum	Mycobacterium microti
	SEQ. NO. 41	Amplification possible	Amplification possible	Amplification possible	Amplification possible
	SEQ. NO. 43			_	
	SEQ. NO. 45	Amplification possible	No amplification	No amplification	No amplification
10	SEQ. NO. 47				
	SEQ. NO. 49	No amplification	Amplification possible	No amplification	No amplification
	SEQ. NO. 51		_		
15	SEQ. NO. 45	No amplification	No amplification	Amplification possible	Amplification possible
	SEQ. NO. 53				
	SEQ. NO. 55	No amplification	no amplification	no amplification	Amplification possible
20	SEQ. NO. 57			l	

EXAMPLE 5

[0068] A 10 ng portion of purified DNA was prepared from each of 4 tubercle bacilli group bacterial species. PCR was carried out using these DNA samples as templates, and the oligonucleotides represented by SEQUENCE NO. 41 and SEQUENCE NO. 43 as primers. The PCR amplification conditions are as described in Example 3. The products amplified by PCR were digested with restriction enzymes Rsa I and Taq 1, and the thus formed DNA fragments were analyzed by an agarose gel electrophoresis. The results are shown in Fig. 16. In this connection, the relationship between lanes and bacterial species is as follows.

Lane 1: Mycobacterium tuberculosis

Lane 2:

Mycobacterium bovis

Lane 3: Lane 4: Mycobacterium africanum Mycobacterium microti

EXAMPLE 6

[0069] Using the oligonucleotides represented by SEQUENCE NO. 1, SEQUENCE NO. 43, SEQUENCE NO. 45, SEQUENCE NO. 47, SEQUENCE NO. 49, SEQUENCE NO. 51, SEQUENCE NO. 53, SEQUENCE NO. 55 and SEQUENCE NO. 57 as primers, PCR was carried out on a solution of disrupted cells of a strain KPM KY631 isolated from a clinical patient of tuberculosis. When the product amplified by PCR was analyzed by an agarose gel electrophoresis, the amplified product was observed only by the combination of SEQUENCE NO. 1 and SEQUENCE NO. 43 and of SEQUENCE NO. 45 and SEQUENCE NO. 47, so that the strain KPM KY631 was identified as the tubercle Mycobacterium tuberculosis (Table 4 and Fig. 15).

EXAMPLE 7

[0070] Using the oligonucleotides represented by SEQUENCE NO. 41 and SEQUENCE NO. 43 as primers, PCR was carried out on a solution of disrupted cells of a strain KPM KY590 isolated from a clinical patient of tuberculosis. When nucleotide sequence of the thus amplified DNA fragment was determined, the thus obtained nucleotide sequence coincided with the nucleotide sequence of the tubercle *Mycobacterium tuberculosis*, so that the strain KPM KY590 was identified as the tubercle *Mycobacterium tuberculosis* (Figs. 1-11).

EXAMPLE 8

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[0071] Using the oligonucleotides represented by SEQUENCE NO. 41 and SEQUENCE NO. 43 as primers, PCR was carried out on a solution of disrupted cells of a strain isolated from a bovine patient of tuberculosis. When the product amplified by PCR was digested with restriction enzymes Rsa I and Taq I and the thus formed DNA fragments were

analyzed by an agarose gel electrophoresis, the result coincided with the pattern obtained from *Mycobacterium bovis*, so that this strain was identified as *Mycobacterium bovis* (Fig. 16).

[0072] The present invention realizes accurate classification and identification of slow growing mycobacteria which are difficult to identify by conventional methods. It also renders possible quick identification of certain species of atypical mycobacteria, such as *Mycobacterium kansasii* and *Mycobacterium gastri*, which are difficult to distinguish by the identification method based on 16S rRNA gene sequence. The present invention is useful in the fields of medical science, immunology, veterinary science, etc.

SEQUENCE LISTING

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			355					360	•	•			305				
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	Ile 385	Asp	Arg	Val	Leu	199 190	Asn	Thr	GTu	Val	G1n 395	Ala	Ile	Ile	Thr	Ala 400	
o .	Leu	G1 y	Thr	6 1y	11e 405	His	Asp	Glu	Phe	Asp 410	Ile	Thr	Lys	Leu	Arg 415	Tyr	
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o .	gtg Val	gtt Val	aac Asn	gcg Ala 20	cta Leu	tcc Ser	acc Thr	cgg Arg	ctc Leu 25	gaa Glu	gtc Val	gag Glu	atc Ile	aag Lys 30	cgc Arg	gac Asp	96
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5	aag Lys	caa Gln 50	61 y 999	gcg Ala	ccg Pro	acc Thr	aag Lys 55	aag Lys	acg Thr	999 Gly	tca Ser	acg Thr 60	gta Val	cgg Årg	ttc Phe	tgg Trp	192
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	aac Asn	ctg Leu	acc Thr	gac Asp 100	gag Glu	agg Arg	gtg Val	acc Thr	caa 61n 105	gac Asp	gag Glu	gtc Val	gtc Val	gac Asp 110	gaa Glu	gtg Val	336
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_	gaa 61u	tcc Ser 130	act Thr	gca Ala	ccg Pro	cac His	aaa Lys 135	gtt Val	aag Lys	agc Ser	cgc Arg	acc Thr 140	ttt Phe	cac His	tat Tyr	ccg Pro	432
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15	gtg Val	gag 61u	atc Ile	gcg Ala 180	atg Met	caa Gln	tgg Trp	aac Asn	gcc Ala 185	999 Gly	tat Tyr	tcg Ser	gag Glu	tcg Ser 190	gtg Val	cac ' His	576
	acc Thr	ttc Phe	gcc Ala 195	aac Asn	acc Thr	atc .Ile	aac Asn	acc Thr 200	cac His	gag Glu	ggc Gly	ggc	acc Thr 205	cac His	gaa Glu	gag Glu	624
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25	cgc Arg 225	aag Lys	cta Leu	ctg Leu	aag Lys	gac Asp 230	aag Lys	gac Asp	ccc Pro	aac Asn	ctc Leu 235	acc Thr	ggt Gly	gac Asp	gat Asp	atc Ile 240	720
30	cgg	gaa Glu	ggc Gly	ctg Leu	gcc Ala 245	gct Ala	gtg Val	atc Ile	tcg Ser	gtg Val 250	aag Lys	gtc Val	agc Ser	gaa Glu	ecg Pro 255	cag Gln	768
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40	aac Asn	ecc Pro 290	acc Thr	gac Asp	tcg Ser	aaa Lys	gtc Val 295	gtt Val	gtg Val	aac Asn	aag Lys	gct Ala 300	gtg Val	tcc Ser	tcg Ser	gcg Ala	912
45		gcc Ala															960
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50	tcc Ser	acg Thr	gat Asp	ccg Pro 340	Arg	aag Lys	tcc Ser	gaa Glu	ctg Leu 345	tat Tyr	gtc Val	gta Val	gaa Glu	99t Gly 350	gac Asp	tcg Ser	1056

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	ctt Leu	ccg Pro 370	ctg Leu	cgc Arg	ggc Gly	aag Lys	atc Ile 375	atc Ile	aat Asn	gtg Val	gag Glu	aaa Lys 380	gcg Ala	cgc Arg	atc Ile	gac Asp	1152
10	cgg Arg 385	gtg Val	cta Leu	aag Lys	aac Asn	acc Thr 390	gaa Glu	gtt Val	cag 61n	gcg Ala	atc Ile 395	atc Ile	acg Thr	gcg Ala	ctg Leu	ggc Gly 400	1200
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	G1y	Tyr	G1u 35	Trp	Ser	61n	Val	Tyr 40	61u	Lys	Ser	Glu	Pro 45	Leu	Gly	Leu	
35	Lys	6] n 50	Gly	Ala	Pro	Thr	Lys 55	Lys	Thr	€1 y	Ser	Thr 60	Val	Arg	Phe	Trp	
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50	Glu	Ser 130	Thr	Ala	Pro	His	Lys 135	Val	Lys	Ser	Arg	Thr 140	Phe	His	Tyr	Pro	
	61y 145	Gly	Leu	Val	Asp	Phe 150	Val	Lys	His		Asn 155	Arg	Thr	Lys	Asn	Ala 160	

· 55

Ile His Ser Ser Ile Val Asp Phe Ser Gly Lys Gly Thr Gly His Glu 165 170 175 Val Glu Ile Ala Het Gln Trp Asn Ala Gly Tyr Ser Glu Ser Val His 185 190 Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His Glu Glu 195 200 205 Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala Lys Asp 210 220 Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp Asp Ile 225 230 235 Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu Pro Gln 245 250 255 Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val Lys Ser 260 265 270 Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe Glu Ala 275 280 285 Asn Pro Thr Asp Ser Lys Val Val Val Asn Lys Ala Val Ser Ser Ala 290 295 300 Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys 305 310 315 Ser Ala Thr Asp Ile Gly Gly Leu Pro Gly Lys Leu Ala Asp Cys Arg 325 330 335 Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly Asp Ser 340 345 350 Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln Ala Ile 355 360 365 Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg Ile Asp 370 380 Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala Leu Gly 385 390 395 400 Thr Gly Ile His Asp Glu Phe Asp Ile Gly Lys Leu Arg Tyr His Lys
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410
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15	cgt gac ggc cac aag tgg tcg cag ttc tac aac aag gcc gtg ccg ggc 144 Arg Asp Gly His Lys Trp Ser Gln Phe Tyr Asn Lys Ala Val Pro Gly 35 40 45
20	acg ctc aaa cag ggt gaa gcc act aag aaa acc gga acg aca att agg 192 Thr Leu Lys Gln Gly Glu Ala Thr Lys Lys Thr Gly Thr Thr Ile Arg 50 55 60
<i>25</i>	tte tgg gcc gac ccg gac atc ttc gag acc acc gaa tac gac ttc gag Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu 65 70 75 80
	acc gtg gca cgc cgg ctg cag gaa atg gca ttc ctg aac aag ggc ttg Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu 85 90 95
<i>30</i>	ace ate aac etc ace gae gag ega gtt gee eag gae gag gtt gte gae 336 Thr Ile Asn Leu Thr Asp Glu Arg Val Ala Gln Asp Glu Val Val Asp 100 105 110
35	gag stc stc agc sac acc scc sag sca ccc aas tcc scc saa saa aas 384 Glu Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Glu Lys 125
	gcg gcc gaa tcc aaa ggg ccg cat aag gtt aag cac cgc act ttc cat Ala Ala Glu Ser Lys Gly Pro His Lys Val Lys His Arg Thr Phe His 130 135 140
40	tac ccc ggc ggg ctg atc gac ttc gtc aag cac atc aac cgg acc aag Tyr Pro Gly Gly Leu Ile Asp Phe Val Lys His Ile Asn Arg Thr Lys 145 150 155 160
4 5	ago cog ato cag cag agt gto gto gco tto gao ggo aag ggt gaa ggg 528 Ser Pro Ile Gln Gin Ser Val Val Ala Phe Asp Gly Lys Gly Glu Gly 165 170 175
50	cac gag gtc gag atc gcg atg cag tgg aac ggc ggc tat tcg gag tcg 576 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 185 190
	gtg cac acc ttc gcc aac acc atc aac acc cac gag ggc ggc acc cac 624

	Va1	His	Thr 195	Phe	Ala	Asn 	Thr	Ile 200	Asn	Thr	His	Glu	61 y 205	Gly	Thr	His	
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	gac Asp	att Ile	cgc Arg	gag Glu	ggc Gly 245	ctg Leu	gcc Ala	gcg Ala	gtc Val	atc Ile 250	tcg Ser	gtg Val	aaa Lys	gtt Val	gcc Ala 255	gaa Glu	768
15	ccg Pro	cag Gln	ttc Phe	gag 61u 260	ggc 61 y	cag Gln	acc Thr	aag Lys	acc Thr 265	aaa Lys	ctg Leu	ggt Gly	aac Asn	acc Thr 270	gju gag	gtc Val	816
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10	Glu Ala Asn Pro 290	Ser Glu Ala Lys Thr 295	Val Val Asn Lys Ala 300	Val Ser
	Ser Ala Gln Ala 305	a Arg Ile Ala Ala Arg 310	Lys Ala Arg Glu Leu 315	Val Arg 320
15	Arg Lys Ser Ala	Thr Asp Leu Gly Gly 325	Leu Pro Gly Lys Leu 330	Ala Asp 335
	Cys Arg Ser Thr 340	Asp Pro Arg Lys Ser	Glu Leu Tyr Val Val 350	Glu Gly
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	Ala Ile Leu Pro 370	leu Arg Gly Lys Ile 375	Ile Asn Val Glu Lys 380	Ala Arg
25	Ile Asp Arg Val 385	Leu Lys Asn Thr Glu 390	Val Gln Ala Ile Ile 395	Thr Ala 400
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	His Lys Ile Val	l Leu)		
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	gtg tcg gtg gtc Val Ser Val Val 20	c aac gcg ttg tcg acc l Asn Ala Leu Ser Thr 25	cgg ctc gag gtg gat Arg Leu Glu Val Asp 30	gtc gcc 96 Val Ala
50	cgc gac ggc tac Arg Asp Gly Tyr	c atg tgg tca cag ttc r Met Trp Ser Gln Phe	tac gat cac gcc gag Tyr Asp His Ala Glu	ccg gga 144 Pro Gly
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	acg Thr	gtg Val	gcg Ala	cgc Arg	cga Arg 85	ctg Leu	cag Gln	gaa Glu	atg Met	gcg Ala 90	ttc Phe	ctg Leu	aac Asn	aag Lys	ggt Gly 95	ttg Leu	288
15	acg Thr	atc Ile	aac Asn	ctc Leu 100	acc Thr	gac Asp	gag Glu	cgg Arg	gtc Val 105	agt Ser	gaa Glu	gag Glu	gag Glu	gtc Val 110	gtc Val	gac Asp	336
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50	ccg Pro	caa Gln	ttc Phe	gag Glu	ggc Gly	cag Gln	acc Thr	aaa Lys	acc Thr	aag Lys	ctg Leu	ggc Gly	aac Asn	acc Thr	g Ju gag	gtc Val	816

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	tcg gcc cag Ser Ala Gln 305.	gcc cga atc gca Ala Arg Ile Ala 310	gcg cgc aag gcg cga Ala Arg Lys Ala Arg 315	gaa ctg gtg cgc 960 Glu Leu Val Arg 320
15	cgc aag agc Arg Lys Ser	gcc acc gac ctc Ala Thr Asp Leu 325	ggt ggg ctg ccg ggt Gly Gly Leu Pro Gly 330	aag ctc gca gac · 1008 Lys Leu Ala Asp 335
20	tgc cgc tcc Cys Arg Ser	acc gac ccg cga Thr Asp Pro Arg 340	aag teg gaa etg tat Lys Ser Glu Leu Tyr · 345	gtg gtg gag ggt . 1056 Val Val Glu Gly 350
	gac tcg gcc Asp Ser Ala 355	ggc ggc tcg gcc Gly Gly Ser Ala	aag agc ggc cgc gac Lys Ser Gly Arg Asp 360	tcg atg ttc cag 1104 Ser Met Phe Gln 365
25	gcg atc ctc Ala Ile Leu 370	ccg ctg cgt ggc Pro Leu Arg Gly 375	aag atc atc aac gtc Lys Ile Ile Asn Val 380	gag aag gcg cgc 1152 Glu Lys Ala Arg
30	atc gac cgg Ile Asp Arg 385	gtg ctg aag aac Val Leu Lys Asn 390	acc gaa gtt cag gcg Thr Glu Val Gln Ala 395	atc atc acc gcg 1200 Ile Ile Thr Ala 400
35	ctg ggc acg Leu Gly Thr	ggg att cac gac Gly Ile His Asp 405	gag ttc gac atc acc Glu Phe Asp Ile Thr 410	aag ctc cgg tac 1248 Lys Leu Arg Tyr 415
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	Arg Asp Gly	Tyr Met Trp Ser	Gln Phe Tyr Asp His	Ala Glu Pro Gly

35 45 Thr Leu Lys Gln Gly Glu Ala Thr Lys Thr Thr Gly Thr Thr Ile Arg 50 60 Phe Trp Ala Asp Pro Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu 65 70 75 80 Thr Val Ala Arg Arg Leu Gln Glu Met Ala Phe Leu Asn Lys Gly Leu 85 90 95 Thr Ile Asn Leu Thr Asp Glu Arg Val Ser Glu Glu Glu Val Val Asp 100 105 110 Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Val Glu Lys 115 120 125 Ala Ala Glu Ser Thr Gly Pro His Lys Val Lys His Arg Thr Phe His 130 140 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys 145 150 155 160 Asn Pro Ile His Asn Ser Ile Val Asp Phe Ser. Gly Lys Gly Pro Gly 175 His Glu Val Glu Ile Ala Met Gln Trp Asn Ala Gly Tyr Ser Glu Ser 180 190 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His 195 200 205 Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 220 Lys Asp Arg Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp 225 235 240 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu 245 250 Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val 260 270 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285 Glu Ala Asn Pro Ala Asp Ala Lys Thr Val Val Asn Lys Ala Val Ser 290 . 295 300 Ser Ala Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg 305 310 315 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 335

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	acg gtg g Thr Val A	gcg cgc cgg Ala Arg Arg 85	ctg cag gas Leu 61n 61u	atg gcg ttc Met Ala Phe 90	ctc aac aac Leu Asn Lys	ggg ttg 288 Gly Leu 95
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•	gcg gcg Ala Ala 130	gaa tcg Glu Ser	act gcg Thr Ala	Pro 135	cat His	aag Lys	gtt Val	aag Lys	cac His 140	cgc Arg	acc Thr	ttc Phe	cac His	432
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20	Val His	acc ttc Thr Phe 195	Ala Asn	Thr	11e 200	Asn	Thr	His	61v	Gly 205	Gly	Thr	His '	624
25	gaa gag Glu Glu 210	ggc ttc Gly Phe	cgc agc Arg Ser	gcg Ala 215	ctg Leu	acg Thr	tcg Ser	gtg Val	gtg Val 220	aac Asn	aag Lys	tac Tyr	gcc Ala	672
30	aaa gac Lys Asp 225	aag aag Lys Lys	ttg ctg Leu Leu 230	aaa Lys	gac : Asp i	aag g Lys /	Asp	ccg Pro 235	aac Asn	ctc Leu	acc Thr	ggc Gly	gac Asp 240	720
	gac att Asp Ile	cgc gaa Arg Glu	ggc ctg Gly Leu 245	gcc (Ala	gcg g Ala V	gtg Val	atc Ile 250	tcg Ser	gtc Val	aag Lys	gtc Val	agc Ser 255	gaa Glu	768
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	tcg gcg Ser Ala 305	cag gcc Gln Ala	cgg atc Arg Ile 310	gcc g	gcg c	ogo a Arg (Lys	gcg Ala 315	cga Arg	gag Glu	ttg Leu	gtg Val	cgt Arg 320	960
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10	gcc Ala	atc Ile 370	ctg Leu	ccg Pro	ctg Leu	cgc Arg	ggc 61y 375	aag Lys	atc Ile	atc Ile	aac Asn	gtc Val 380	gag Glu	aag Lys	gcc Ala	cgc Arg	1152
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50	Thr :	Ile	Asn	Leu 100	Thr	Asp	Glu	Arg	Va1 105	Ser	Asn	Glu	Glu	Val 110	Val.	Asp	
55				_		_				_					G1 u		

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Ala Ala Glu Ser Thr Ala Pro His Lys Val Lys His Arg Thr Phe His 130 140 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys 145 150 160 Ser Pro Ile Gln Gln Ser Ile Ile Asp Phe Asp Gly Lys Gly Pro Gly 165 170 175 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 180 190 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His 195 200 205 Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 215 220 Lys Asp Lys Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp 225 230 235 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Lys Val Ser Glu 245 250 255. Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val 260 270 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285 Glu Ala Asn Pro Ala Asp Ala Lys Val Val Val Asn Lys Ala Val Ser 290 300 Ser Ala 61n Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg 305 310 315 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 335 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly 340 350 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln 355 360 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 370 375 380 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr 405 415 His Lys Ile Val Leu 420

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50	aac ccc atc cac cag agc atc atc gat ttc ggt ggg aag ggc ccc ggc 528 Asn Pro Ile His Gln Ser Ile Ile Asp Phe Gly Gly Lys Gly Pro Gly 165 170 175

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1248

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	Ala	11e 370	Leu	Pro	Leu	Arg	G1y 375	Lys	Ile	Ile	Asn	Va1 380	Glu	Lys	Ala	Arg	
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	Gln Ala Arg Ile Ala Ala Arg Lys Ala Arg Glu Leu Val Arg Arg Lys 305 310 320
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J							gag Glu										336
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50	Phe Trp Ala Asp Ser Asp Ile Phe Glu Thr Thr Glu Tyr Asp Phe Glu 65 70 75 80
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Thr Ile Asn Leu Thr Asp Glu Arg Val Thr Pro Asp Glu Val Val Asp 100 105 Asp Val Val Ser Asp Thr Ala Glu Ala Pro Lys Ser Ala Glu Lys 115 120 125 Ala Ala Glu Ser Thr Ala Pro His Lys Val Lys Ser Arg Thr Phe His 130 140 Tyr Pro Gly Gly Leu Val Asp Phe Val Lys His Ile Asn Arg Thr Lys 145 150 155 160 Ser Pro Ile Gln Gln Ser Ile Val Asp Phe Glu Gly Lys Gly Ser Gly 175 His Glu Val Glu Ile Ala Met Gln Trp Asn Gly Gly Tyr Ser Glu Ser 185 190 Val His Thr Phe Ala Asn Thr Ile Asn Thr His Glu Gly Gly Thr His 195 200 205 Glu Glu Gly Phe Arg Ser Ala Leu Thr Ser Val Val Asn Lys Tyr Ala 210 215 220 Lys Asp Lys Lys Leu Leu Lys Asp Lys Asp Pro Asn Leu Thr Gly Asp 225 230 235 Asp Ile Arg Glu Gly Leu Ala Ala Val Ile Ser Val Arg Val Ala Glu 245 250 255 Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val 260 270 Lys Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285 Glu Ala Asn Pro Ser Glu Ala Lys Thr Ile Val Asn Lys Ala Val Ser 290 295 300 Ser Ala Gln Ala Arg Leu Ala Ala Arg Lys Ala Arg Glu Leu Val Arg 305 310 315 320 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 335 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly 340 345 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln 355 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 370 380 Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala

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***	aac ctg acc gad Asn Leu Thr Asp 100	Glu Arg Val	acc caa gac ga Thr Gln Asp Gli 105	g gtc gtc gac g u Val Val Asp G 110	aa gtg 336 lu Val
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	G1 y 145	61 <i>y</i>	Leu	Val	Asp	Phe 150	Val	Lys	His	Ile	Asn 155		Thr	Lys	Asn	Ala 160	
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		Leu Arg	Gly Lys	Ile 375	Ile A	sn Val			Ąŗg	Ile	Asp	
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50	Ile His	Ser Ser	Ile Val 165	Asp	Phe S	er Gly 170	Lys (Gly Thr	Gly	His 175	Glu	
	Val Glu	lle Ala 180	Met Gln	Trp		la Gly 85	Tyr :	Ser Glu	Ser 190	Va1	His	

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Pro Gln Phe Glu Gly Gln Thr Lys Thr Lys Leu Gly Asn Thr Glu Val 265 270 Ser Phe Val Gln Lys Val Cys Asn Glu Gln Leu Thr His Trp Phe 275 280 285 Glu Ala Asn Pro Ala Asp Ala Lys Val Ile Val Asn Lys Ala Val Ser 290 295 300 Ser Ala Gin Ala Arg Ile Ala Ala Arg Lys Ala Arg Giu Leu Val Arg 305 310 315 Arg Lys Ser Ala Thr Asp Leu Gly Gly Leu Pro Gly Lys Leu Ala Asp 325 330 Cys Arg Ser Thr Asp Pro Arg Lys Ser Glu Leu Tyr Val Val Glu Gly 340 350 Asp Ser Ala Gly Gly Ser Ala Lys Ser Gly Arg Asp Ser Met Phe Gln 355 360 365 Ala Ile Leu Pro Leu Arg Gly Lys Ile Ile Asn Val Glu Lys Ala Arg 370 380 Ile Asp Arg Val Leu Lys Asn Thr Glu Val Gln Ala Ile Ile Thr Ala 385 390 395 400 Leu Gly Thr Gly Ile His Asp Glu Phe Asp Ile Thr Lys Leu Arg Tyr 405 410 415 His Lys Ile Val Leu 420 <210> 39 <211> 1257 <212> DNA <213> Mycobacterium kansasii <220> <221> CDS <222> (1)..(1257) <400> 39 tcc gac gcc tac gcg ata tcg ggc ggg ctg cac ggt gtg ggt gtc tcg Ser Asp Ala Tyr Ala Ile Ser Gly Gly Leu His Gly Val Gly Val Ser 1 5 10 48 gtg gtc aac gca ctg tcc acc cgg ctg gag gtg gag atc aag cgc gac Val Val Asn Ala Leu Ser Thr Arg Leu Glu Val Glu Ile Lys Arg Asp 25 30 96 ggc cat gag tgg tcg cag gtt tac gag aaa tcc gag ccg atg gga ctc Gly His Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Met Gly Leu 35 40 144

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15	aat Asn	ctg Leu	acc Thr	gat Asp 100	cag Gln	cgg Arg	gtg Val	acc Thr	cag Gln 105	gac Asp	gag Glu	gtc Vai	gtc Val	gac Asp 110	gag Glu	gtg Val	336
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50	Gly His Glu Trp Ser Gln Val Tyr Glu Lys Ser Glu Pro Met Gly Leu 35 40 45	

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Claims

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- 1. A method for identifying a slow growing mycobacteria species, which comprises amplifying the regions corresponding to SEQUENCE NO. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 in the DNA which encodes DNA gyrase β subunit of a slow growing mycobacteria in a sample, determining and comparing the nucleotide sequence of the amplified fragment with the nucleotide sequences described in SEQUENCE NO. 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, thereby calculating a genetic distance of the sequence of the amplified fragment from each sequence, and identifying the species of the slow growing mycobacteria in a sample based on the genetic distance.
- 2. A method for detecting Mycobacterium kansasii, which comprises detecting Mycobacterium kansasii using, as a primer or probe, an oligonucleotide which comprises a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 4, or a complementary sequence thereof, and substantially functions as a primer or probe.
- 3. A Mycobacterium kansasii detection kit which comprises an oligonucleotide which comprises a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 4, or a complementary sequence thereof, and substantially functions as a primer or probe.
- 4. A method for detecting Mycobacterium gastri, which comprises detecting Mycobacterium gastri using, as a primer or probe, an oligonucleotide which comprises a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 6, or a complementary sequence thereof, and substantially functions as a primer or probe.
- 5. A Mycobacterium gastri detection kit which comprises an oligonucleotide which comprises a sequence coding for a part of or the entire portion of the amino acid sequence described in SEQUENCE NO. 6, or its complementary sequence, and substantially functions as a primer or probe.
- A method for identifying a slow growing mycobacteria species, which comprises

checking, in DNA of a sample, the existence of a DNA comprising a unique region having a different nucleotide sequence in the DNA sequence coding for DNA gyrase β subunit among slow growing mycobacteria. identifying a bacterium in a sample based on the existence of said DNA as a marker.

The method for identifying a slow growing mycobacteria species according to claim 6, wherein the slow growing mycobacteria are Mycobacterium simiae, Mycobacterium bovis, Mycobacterium szuigai, Mycobacterium maimoense, Mycobacterium intracellulare, Mycobacterium avium, Mycobacterium gordonae, Mycobacterium africa-

num, Mycobacterium tuberculosis, Mycobacterium gastri, Mycobacterium marinum, Mycobacterium microti, Mycobacterium asiaticum, Mycobacterium scrofulaceum, Mycobacterium branderi, Mycobacterium paratuberculosis, and Mycobacterium kansasii.

5 8. The method for identifying a slow growing mycobacteria species according to claim 6, which comprises the following steps (1) to (4):

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- (1) synthesizing an oligonucleotide which comprises a unique region having a different nucleotide sequence in the DNA sequence coding for DNA gyrase β subunit among slow growing mycobacteria,
- (2) preparing a solution which comprises the oligonucleotide synthesized in the step (1), dNTP, DNA polymerase and a bacterial DNA in a sample,
- (3) heating the solution prepared in the step (2) repeatedly under such conditions that polymerase chain reaction can occur, and
- (4) subjecting the solution obtained in the step (3) to electrophoresis to identify the species of the bacterium in a sample based on the electrophoresis pattern.
- The method for identifying a slow growing mycobacteria species according to claim 8, wherein the oligonucleotide is an oligonucleotide which encodes the amino acid sequence described in SEQUENCE NO. 46, SEQUENCE NO. 48, SEQUENCE NO. 50, SEQUENCE NO. 52, SEQUENCE NO. 54, SEQUENCE NO. 56 or SEQUENCE NO. 58.
- 10. The method for identifying a slow growing mycobacteria species according to claim 8, wherein the oligonucleotide is an oligonucleotide represented by SEQUENCE NO. 45, SEQUENCE NO. 47, SEQUENCE NO. 49, SEQUENCE NO. 51, SEQUENCE NO. 53, SEQUENCE NO. 55 or SEQUENCE NO. 57.
- 25 11. The method for identifying a slow growing mycobacteria species according to claim 6, which comprises the following steps (1) to (4):
 - (1) synthesizing a first oligonucleotide which is identical to a first partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria and a second oligonucleotide which is complementary to a second partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria, said first partial sequence and said second partial sequence being respectively conserved among slow growing mycobacteria,
 - (2) subjecting the two oligonucleotides synthesized in the step (1) as primers and a bacterial DNA sample as a template to the polymerase chain reaction,
 - (3) mixing the DNA fragment amplified in the step (2) with a restriction enzyme under the conditions at which the restriction enzyme is active, said restriction enzyme recognizing the sequence unique to one or more slow growing mycobacteria, and
 - (4) subjecting the mixture obtained in the step (3) to electrophoresis to identify the species of the bacterium in a sample based on the electrophoresis pattern.
 - 12. The method for identifying a slow growing mycobacteria species according to claim 11, wherein the two oligonucleotides to be used as primers are oligonucleotides represented by SEQUENCE NO. 1 and SEQUENCE NO. 3, and the restriction enzymes to be used are Rsa I and Taq I.
- 45 13. An identification kit for a slow growing mycobacteria species, which comprises an oligonucleotide containing a region of DNA coding for DNA gyrase β subunit, a region having different nucleotide sequence among slow growing mycobacteria.
 - 14. An identification kit for a slow growing mycobacteria species, which comprises
 - a first oligonucleotide which is identical to a first partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria,
 - a second oligonucleotide which is complementary to a second partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria, said first partial sequence and said second partial sequence being respectively conserved among slow growing mycobacteria, and
 - one or more restriction enzyme recognizing the sequence unique to one or more slow growing mycobacteria.
 - 15. A method for detecting a slow growing mycobacteria species, which comprises

checking, in DNA of a sample, the existence of a DNA comprising a unique region having a different nucleotide sequence in the DNA sequence coding for DNA gyrase β subunit among slow growing mycobacteria, detecting a bacterium in a sample based on the existence of said DNA as a marker.

- 16. The method for detecting a slow growing mycobacteria species according to claim 15, wherein the slow growing mycobacteria are Mycobacterium similae, Mycobacterium bovis, Mycobacterium szulgal, Mycobacterium malmoense, Mycobacterium intracellulare, Mycobacterium avium, Mycobacterium gordonae, Mycobacterium africanum, Mycobacterium tuberculosis, Mycobacterium gastri, Mycobacterium marinum, Mycobacterium microti, Mycobacterium aslaticum, Mycobacterium scrofulaceum, Mycobacterium branderi, Mycobacterium paratuberculosis, and Mycobacterium kansasii.
 - 17. The method for detecting a slow growing mycobacteria species according to claim 15, which comprises the following steps (1) to (4):

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- (1) synthesizing an oligonucleotide which comprises a unique region having a different nucleotide sequence in the DNA sequence coding for DNA gyrase β subunit among slow growing mycobacteria,
- (2) preparing a solution which comprises the oligonucleotide synthesized in the step (1), dNTP, DNA polymerase and a bacterial DNA in a sample,
- (3) heating the solution prepared in the step (2) repeatedly under such conditions that polymerase chain reaction can occur, and
- (4) subjecting the solution obtained in the step (3) to electrophoresis to detecting the bacterium in a sample based on the electrophoresis pattern.
- 18. The method for detecting a slow growing mycobacteria species according to claim 17, wherein the oligonucleotide is an oligonucleotide which encodes the amino acid sequence described in SEQUENCE NO. 46, SEQUENCE NO. 48, SEQUENCE NO. 50, SEQUENCE NO. 52, SEQUENCE NO. 54, SEQUENCE NO. 56 or SEQUENCE NO. 58.
 - 19. The method for detecting a slow growing mycobacteria species according to claim 17, wherein the oligonucleotide is an oligonucleotide represented by SEQUENCE NO. 45, SEQUENCE NO. 47, SEQUENCE NO. 49, SEQUENCE NO. 51, SEQUENCE NO. 53, SEQUENCE NO. 55 or SEQUENCE NO. 57.
 - 20. The method for detecting a slow growing mycobacteria species according to claim 15, which comprises the following steps (1) to (4):
 - (1) synthesizing a first oligonucleotide which is identical to a first partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria and a second oligonucleotide which is complementary to a second partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria, said first partial sequence and said second partial sequence being respectively conserved among slow growing mycobacteria,
 - (2) subjecting the two oligonucleotides synthesized in the step (1) as primers and a bacterial DNA sample as a template to the polymerase chain reaction.
 - (3) mixing the DNA fragment amplified in the step (2) with a restriction enzyme under the conditions at which the restriction enzyme is active, said restriction enzyme recognizing the sequence unique to one or more slow growing mycobacteria, and
 - (4) subjecting the mixture obtained in the step (3) to electrophoresis to detect the bacterium in a sample based on the electrophoresis pattern.
- 21. The method for detecting a slow growing mycobacteria species according to claim 20, wherein the two oligonucleotides to be used as primers are oligonucleotides represented by SEQUENCE NO. 1 and SEQUENCE NO. 3, and the restriction enzymes to be used are Rsa I and Taq I.
 - 22. A detection kit for a slow growing mycobacteria species, which comprises an oligonucleotide containing a region of DNA coding for DNA gyrase β subunit, a region having different nucleotide sequence among slow growing mycobacteria.
 - 23. A detection kit for a slow growing mycobacteria species, which comprises
 - a first oligonucleotide which is identical to a first partial sequence in the DNA sequence coding for the DNA

gyrase β subunit of slow growing mycobacteria,

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a second oligonucleotide which is complementary to a second partial sequence in the DNA sequence coding for the DNA gyrase β subunit of slow growing mycobacteria, said first partial sequence and said second partial sequence being respectively conserved among slow growing mycobacteria, and

one or more restriction enzyme recognizing the sequence unique to one or more slow growing mycobacteria.

- 24. A method for identifying a slow growing mycobacteria species, which comprises the following steps (1) to (4):
 - (1) synthesizing an oligonucleotide which comprises a sequence corresponding to a region in the DNA gyrase β subunit wherein the 3'-side nearest neighbor base to said region in the DNA gyrase β subunit is a unique base among the slow glowing mycobacteria,
 - (2) preparing a solution which comprises the oligonucleotide synthesized in the step (1), a labeled ddNTP, DNA polymerase, and a bacterial DNA in a sample,
 - (3) heating the solution prepared in the step (2) under such conditions that reaction between the labeled ddNTP and the oligonucleotide occurs,
 - (4) checking the existence of the labeled oligonucleotide, and
 - (5) identifying a bacterium in a sample based on the existence of the labeled oligonucleotide.
- 25. A method for detecting a slow growing mycobacteria species, which comprises the following steps (1) to (4):
 - (1) synthesizing an oligonucleotide which comprises a sequence corresponding to a region in the DNA gyrase β subunit wherein the 3'-side nearest neighbor base to said region in the DNA gyrase β subunit is a unique base among the slow glowing mycobacteria,
 - (2) preparing a solution which comprises the oligonucleotide synthesized in the step (1), a labeled ddNTP, DNA polymerase, and a bacterial DNA in a sample,
 - (3) heating the solution prepared in the step (2) under such conditions that reaction between the labeled ddNTP and the oligonucleotide occurs,
 - (4) checking the existence of the labeled oligonucleotide, and
 - (5) detecting a bacterium in a sample based on the existence of the labeled oligonucleotide.

Fig. 1	·
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ATCC25274	GGTGAGAACAGCGGCTACACCGTCAGCGGTGGGCTGCACGGTGTCGGTGTCAGTGGTC
KPM1403	GGGGAGAACAGTGGCTACACCGTCAGCGGCGGGTTGCACGGGGTCGGAGTGTCGGTGGTC
KPM2027	GGCGAGAACAGCGGCTACACCGTCAGCGGTGGGTTGCACGGAGTGGGCGTGTCGGTGGTC
KPM1201	GGCGAGAACAGTGGTTACAACGTCAGTGGTCGTCTCCACGCGTGGGTGTGTCGGTGGTC
KPM2403	GGCGAGAACAGTGGCTACAACGTCAGTGGTGGTCGCACGGCGTCGGGGTGTCGGTGGTG
KPM3012	GGCGAGAACAGCGGCTACAACGTCAGTGGTCGCACGCGCCGTCGGCGTCTCGGTGGTC
Bovine10	GGCGAGAACAGCGGCTACAACGTCAGCGGCGGCGTCTGCACGGCGTCGGCGTCTCGGTGGTC
KPM3101	GGTGAGAACAGCGGTTACAACGTCAGCGGTGGCCTGCACGGCGTGGGCGTCTCGGTGGTC
KPM3401	GGCGAGAACAGCGGATACAACGTCAGTGGCGGTTTGCACGGTGTCGGCGTGTCGGTGGTC
ATCC51789 ·	GCGGATGACAGCGCCTACGCGGTCTCGGGGGTGTCGGCGTGTCGGTGGTCGGTGGTC
T801	TCGGACGCGTATGCGATATCTGGTGGTCTGCACGGCGTCGGCGTGTCGGTGGTC
T901	TCGGACGCGTATCCGATATCTGGTGGTCTGCACGGCGTCGGCGTGTCGGTGGTT
T704	TCGGACGCGTATGCGATATCTGGTGGTCTGCACGGCGTCGGCGTGTCGGTGGTT
T021	TOGGACGCGTATGCGATATCTGGTGGTCTGCACGGCGTCGGCGTGTCGGTGGTT
KPM3504	TCCGACGCCTATGCGATATCGGGTGGACTGCACGGTGTGGGTGTCTCGGTGGTC
KPM1001	TCCGACGCCTACGCGATATCGGGCGGGCTGCACGGTGTGGGTGTCTCGGTGGTC 6 0
Sequence	No 41
	* 140· 41 *** ** ** ** ** *** *** *** *** ***
Sequence Sequence	No. 61
Sequence Sequence KPM2201	No. 61: Sequence No. 1
Sequence	No. 61: No. 62: Sequence No. 1 AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG
Sequence KPM2201	NO. 61: Sequence No. 1 AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCGTTGTCGACCCGACTCGAGGTCGACATCAAGCGCGACGGGCACGAGTGGTCCCAG
Sequence KPM2201 ATCC25274	AACGCCTGTCCACCCGCCTGGAAGTCAAACGTCAAGCGTGACGGCACGAGTGGTCCCAG AACGCCTTGTCGACCCCGACTCGAGGTCGACATCAAGCGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCTATGAGTGGTTCCAG
Sequence KPM2201 ATCC25274 KPM1403	No. 61: No. 62: No. 62
Sequence KPM2201 ATCC25274 KPM1403 KPM2027	AACGCGTTGTCCACCCGCCTGGAAGTCACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCGTTGTCGACCCCGCCTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCTATGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAAGTCACCATCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTTCCAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201	No. 61: No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCGTTGTCGACCCGACTCGAGGTCGACATCAAGCGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCTATGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTCGACATCAAGCGTGACGCCCACAAGTGGTCGCAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403	No. 61: No. 62: No. 62: No. 62: No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCGTTGTCGACCCGACTCGAGGTCGACATCAAGCGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTCGACATCAAGCGTGACGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCCCCCGCGACGGCTACGAGTGGTCGCAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012	AACGCGCTGTCCACCCGGCTCGAGGTCAACATCACCGCGCGCCGCGCCACAGTGGTCGCAG AACGCGCTGTCCACCCGCCTGGAGGTCACATCAAACGCGGGCACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCTATGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAAGTCACCTCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCCACCCGGCTCGAGGTCACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10	No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCGTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTCGACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGCTACGAGTGGTCGCAG AACGCGCTGTCCACCCGGCTCGAGGTCAACATCGCCCGCGATGGCTACGAATGGTCGCAG
KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101	No. 62: No. 62: No. 62: No. 62: No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCGTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACCCGGCTCGAGGTCAACATCACCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGATGGCTACGAATGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGCTACCAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGATGTCGCCCGCGACGCTACATTGGTCACAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401	No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCGTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGCGACGGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAAGTCACCTCAAGCGCGACGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGCACAAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTCAACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACCAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACCATGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGAATGTCGCCCGCGACGGCTACCATGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGAGATCGCCCGCGACGGTACGATGGTCACAG AACGCCATTGTCGACTCGAC
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789	ACGCGTTGTCGACCCGGCTCGAGGTCGACATCAACGCGACGGCCACGAGTGGTCCCAG AACGCCTTGTCGACCCGCGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCCACCCGGCTCGAGGTCGACATCAAGCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACATTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGTACGATTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGATTCGCCCGCGACGGTACGAGTGGTTCACAG AACGCGTTGTCCACCCGGCTCGAGGTGGAGTCGACGGTACGAGTGGTCTCAG AACGCGTTATCCACCCGGCTCGAAGTCGAGGTGGACGACGGTACGAGTGGTCTCAG AACGCGTTATCCACCCGGCTCGAAGTCGAGATCGAGGTACGAGTGGTCTCAG
KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801	No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCGTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGCACGAGTGGTTCGCAG AACGCGCTGTCCACCCGGCTCGAGGTCGACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGATGTCGCCCGCGACGGCTACATGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGATTCGCCCGCGACGGTACGAGTGGTTCACAG AACGCGTTATCCACCCGGCTCGAAGTCGAGATCGAGCGGGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG
KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801	ACGCGTTGTCGACCGGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCGTTGTCGACCCGACTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTCCCAG AACGCGCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGCGACGGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGGCTGGAAGTCGACATCAAGCGCGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGGCTCGAGGTCGACATCAAGCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACATGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGAGTTGCCCCGCGACGGCTACATGTGGTCACAG AACGCGTTGTCCACCCGGCTCGAGGTGGAGTTGCGCCCGCGACGGTACGAGTGGTTCCAG AACGCGTTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG
Sequence KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901	No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGTACGAGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGAATCGCCCGCGACGGTACGAGTGGTCTCAG AACGCGTTTGTCGACCCGGCTCGAAGTCGAGTTGAACGCGCGACGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG
KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901 T704 T021	No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCGTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGCCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGGCTCGAGGTCGACATCAAGCGTGACGGCCACAAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTGGACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGAATCGCCCGCGACGGCTACAATGGTCCACG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGCTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGCTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGCTACGAGTGGTCCCAA
KPM2201 ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3401 ATCC51789 T801 T901 T704 T021 KPM3504	No. 62: No. 62: AACGCGTTGTCGACGCGGTTGGAAGTCGACATCAAACGCGACGGGCACGAGTGGTCGCAG AACGCCTTGTCGACCCGACTCGAGGTCGACATCAAACGCGACGGGCACGAGTGGTCCCAG AACGCCCTGTCCACCCGCCTGGAAGTCAACGTCAAGCGTGACGGCACGAGTGGTTCCAG AACGCGCTGTCCACCCGCCTGGAGGTCACCATCAAGCGCGACGGGCACGAGTGGTTTCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGATACGAGTGGTCGCAG AACGCGCTGTCCACCCGACTGGAAGTCGACATCAAGCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCCACTCGGCTCGAGGTCAACATCGCCCGCGACGGCTACGAGTGGTCGCAG AACGCGCTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGCTACGAATGGTCGCAG AACGCGTTGTCGACCCGGCTCGAGGTGGACATCGCCCGCGACGGTACGAGTGGTCACAG AACGCGTTGTCGACCCGGCTCGAGGTGGAATCGCCCGCGACGGTACGAGTGGTCTCAG AACGCGTTTGTCGACCCGGCTCGAAGTCGAGTTGAACGCGCGACGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG AACGCGCTATCCACCCGGCTCGAAGTCGAGATCAAGCGCGACGGGTACGAGTGGTCTCAG

Fig. 2	÷
KPM2201	TATTACAAGCECGCGGTGCCGGGCACCCTCAAGCAGGGTGAGACGACCCGCAAGACCGGC
ATCC25274	TATTACGAGCECGCCGTTCCTGGCACGCTCAAGCAGGGCGAGCGACCAAGAAGACCGGC
KPM1403	TACTACGACCEGGCGETGCCCGGCACCCTCAAGCAAGGCGAGGCG
KPM2027	TACTACGACCECGCCETGCCCGGAACCCTCAAGCAGGGCGAGGCCACCAAGAAGACCGGA
KPM1201	TTCTACGACCGCCCAGCCGGGCACCCTCAAACAGGGCGAGGCAACCAAGAAGACCGGA
KPM2403	TTCTACAACAAGGCCGTGCCGGGCACGCTCAAACAGGGTGAAGCCACTAAGAAAACCGGA
KPM3012	TACTACGACCACGCCGTGCCCGGCACCCTCAAGCAGGGCGAGGCCACCAAGCGCACCGGC
Bovine10	TACTACGACCACGCCGTGCCCGGCACCCTCAAGCAGGGCGAGGCCACCAAGCGCACCGGC
KPM3101	TTCTACGACCACGCCGTACCCGGAACGCTCAAACAGGGTGAGGCCACCAAGCGGACGGGC
KPM3401	TTCTACGATCACGCCGAGCCGGGAACCCTCAAACAGGGCGAGGCCACCAAGACGACGGGA
ATCC51789	CATTACGACCGCTCTGTCCCCGGCACGCTCAAGCAAGGCGAGAAAACCAAAAAGACCGGC
T801	GTTTATGAGAAGTCGGAACCCCTGGGCCTCAAGCAAGGGGCGCCGACCAA <u>GAAGACGGGG</u>
T901	GTTTATGAGAAGTCGGAACCCCTGGGCCTCAAGCAAGGGGCGCCGACCAA <u>GAAGACGGGG</u>
T704	GTTTATGAGAAGTCGGAACCCCTGGGCCTCAAGCAAGGGGCGCCGACCAA <u>GAAGACGGGG</u>
T021	GTTTATGAGAAGTCGGAACCCCTGGGCCTCAAGCAAGGGGCGCCGACCAA <u>GAAGACGGGG</u>
KPM3504	GTTTATGAGAAGTCCGAGCCGATGGGACTCAAGCAAGGCGCGCCGACGAAGAAGACCGGC
KPM1001	GTTTACGAGAAATCCGAGCCGATGGGACTCAAGCAAGGCGCGCCGACTAAGAAGACCGGC 180
	** * * ** ***
	Sequences No. 45, 49
KPM2201	ACCACAATCCGGTTCTGGGCGGATCCGGAGATCTTCGAGACCACCCAATACGACTTCGAG
ATCC25274	ACCACCATCCGGTTCTGGGCGGACCCGGACATCTTCGAGACCACCCAGTACGACTTCGAG
KPM1403	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG
KPM1403 KPM2027	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCGGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG
KPM1403 KPM2027 KPM1201	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCGGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACTCGGACATCTTTGAGACCACCGAATACGACTTCGAG
KPM1403 KPM2027 KPM1201 KPM2403	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACTCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCGGACATCTTCGAGACCACCGAATACGACTTCGAG
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCTCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCTCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Boyine10 KPM3101 KPM3401	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCGGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCTCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACGATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACGATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGGTCCGCTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGGATTACGACTTCGAG TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGGTCCGCTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGGTCCGCTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGGAATACGACTTCGAG TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901	ACCACGATCCGGTTCTGGGCCGACCCGAAATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGGTCCGCTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTACGGT
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901 T704 T021 KPM3504	ACCACGATCCGGTTCTGGGCCGATCCTGAGATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACGATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAGTACGACTTCGAG TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA
KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901	ACCACGATCCGGTTCTGGGCCGACCCGAAATCTTCGAAACCACCCAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGAAATCTTCGAAACCACACAGTACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCGGACATCTTTGAGACCACCGAATACGACTTCGAG ACGACAATTAGGTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGAATACGACTTCGAG ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAA ACCACCATCCGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACCATCAGGTTCTGGGCCGACCCCGACATCTTCGAGACCACCGAGTACGACTTCGAG ACCACGGTCCGCTTCTGGGCCGACCCCGGACATCTTCGAGACCACCGGAATACGACTTCGAA TCAACGGTGCGGTTCTGGGCCGACCCCGCTGTTTTCGAAACCACGGAATACGACTTCGAA TCAACGGTACGGT

Fig. 3	
KPM2201	ACGGTGGCGCGCCGGCTGCAGGAGATGGCGTTCCTGAACAAGGGTCTGACGATCAATCTB
ATCC25274	ACGGTGGCGCGCCCCAAGAGATGGCGTTCCTGAACAAGGGCTTGACCATCAACTTG
KPM1403	ACGGTGGCGCCGGTTGCAGGAAATGGCGTTCCTCAACAAGGGCCTGACCATCAACCTC
KPM2027	ACCOTOGCGGCGGCGGCTGCAGGAGATGGCCTTCCTCAACAAGGGCCTCACCATCAACCTC
KPM1201 .	ACGGTGGCGCGCCCTGCAGGAGATGGCGTTCCTCAACAAGGGCCTGACCATCAACCTC
KPH2403	ACCOTGGCACGCCGGCTGCAGGAAATGGCATTCCTGAACAAGGGCTTGACCATCAACCTC
KPM3012	ACGGTGGCCCGGCGGCTGCAGGAAATGGCGTTCCTCAACAAGGGCCTGACCATCAACCTC
Bovine10	ACGGTGGCCGGCGGCTGCAGGAAATGGCGTTCCTCAACAAGGGCCTGACCATCAACCTC
KP#3101	ACGGTGGCGCCGGCTGCAGGAAATGGCGTTCCTCAACAAGGGGTTGACCATCAACCTC
KPN3401 ·	ACGGTGGCGCCCGACTGCAGGAAATGGCGTTCCTGAACAAGGGTTTGACGATCAACCTC
ATCC51789	ACGETCGCACGCCGGCTGCAGGAAATGGCGTTCCTCAACAAAGGGCTEACCATCAACCTG
T801	ACCGTCGCCCGCCGGCTGCAAGAGATGGCGTTCCTCAACAAGGGGCTGACCATCAACCTG
T901	ACCGTCGCCCGCCGGCTGCAAGAGATGGCGTTCCTCAACAAGGGGCTGACCATCAACCTG
1704	ACCGTCGCCGGCCGGCTGCAAGAGATGGCGTTCCTCAACAAGGGGCTGACCATCAACCTG
T021	ACCET CECCCECCECCTECAAGAGATEGCETTCCTCAACAAGGGGCTGACCATCAACCTE
KPM3504	ACCGTCGCGACGGTTGCAGGAGATGGCGTTTCTCAACAAGGGGCTCACCATCAACCTG
KPM1001	ACCGTCGCACGACGGTTGCAGGAGATGGCGTTTCTCAACAAGGGGCTCACCATCAATCTG 300
	** ** ** ** ** * * * * ** ** ** ** *** *** *** *** *** *** ***
KPM2201	ACCGACGAACGCGTCGAGCAGGACGAGGTTGTCGACGAGGTCGTCAGCGACACCGCCGAA
ATCC25274	ACCGACGAGCGGGTGGACGAGGTCGTCGATGAAGTCGTCAGCGACACCGCCGAT
KP#1403	ACCGACGAACGTGTCGAGCAGGACGAGGTGGTCGATGAGGTGGTTAGCGACACCGCCGAG
KPM2027	ACCGACGAACGAGTGGAGCAGGACGAGGTCGTCGACGAGGTCGTCAGCGACACCGCCGAG
KPW1201	ACCGACGAGCGGGTCACCCCGGACGAGGTCGTCGACGACGTCGTCAGTGATACCGCCGAA
KPM2403	ACCGACGAGCGAGTTGCCCAGGACGAGGTTGTCGACGAGGTCGTCAGCGACACCGCCGAG
KPM3012	ACCGACGAGCGGGTGACCAACGAGGTCGTCGACGAGGTGGTCAGCGACACCGCCGAC
Bovine10	ACCEACGAGCGGTGACCAACEAAGAGGTCGTCGACGAGGTGGTCAGCGACACCGCCGAC
KPM3101	ACCGACGAGCGGTGAGCAACGAGGAGGTCGTCGACGAGGTCGTCAGCGATACCGCCGAC
KPM3401	ACCEACEAGCGGGTCAGTGAAGAGGAGGTCGTCGACGATGTCGTCAGCGACACCGCCGAG
ATCC51789	ACCGACGAGCGGGTGCGAAACGAAGAAGTCGTCGACGAGGTCGTCAGCGACACCGCCGAC
T801	ACCGACGAGAGGGTGACCCAAGACGAGGTCGTCGACGAAGTGGTCAGCGACGTCGCCGAG
T901	ACCGACGAGAGGGTGACCCAAGACGAGGTCGTCGACGAAGTGGTCAGCGACGTCGCCGAG
T704	ACCGACGAGAGGGTGACCCAAGACGAGGTCGTCGACGAAGTGGTCAGCGACGTCGCCGAG
T021	ACCGACGAGAGGGTGACCCAAGACGAGGTCGTCGACGAAGTGGTCAGCGACGTCGCCGAG
KPM3504	ACCEATCAGCGGGTAACCCAGGACGAAGTGGTCGACGAGGTGGTCAGCGACGTCGCCGAG
KPM1001	ACCGATCAGCGGGTGACCCAGGACGAGGTCGTCGACGAGGTGGTCAGCGACGTCGCCGAG 360
	***** * * ** ** ** ** ** *** ***** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

Fig. 4 **GCGCCCAAATCCGCCGAAGAGAAGGCTGCCGAATCCAAGGCCCCGCACAAGGTCAAGCAG** KPM2201 **ECGCCCAAGTCCGCCGAAGAGAAGGCGCCGCAATCCAAAGCGCCGCACAAGGTTAAGCAC** ATCC25274 GCCCCGAAGTCACCCGAGGAGCAGGCGGCCGAATCGGCCAAAGCCCGCACAAGGTCAAGCAC KPM1403 **ECACCEAAGTCCECCEAAGAGAAGGCCGCGGAATCGACTGCGCCACACAAGGTCAAGCAC KPM2027 GCACCAAAGTCCGCCCAGGAGAAGGCCGCCGAATCGACCGCGCCGCACAAGGTCAAGAGC** KPH1201 **ECACCCAAGTCCECCGAAGAAAAGGCGGCCGAATCCAAAGGGCCGCATAAGGTTAAGCAC** KPM2403 GCACCCAAGTCEECECAGGAGAAGGCGGCGGAATCGGCTGCGCCGCATAAGGTCAAGCAC KP#3012 **GCACCCAAGTCGGCGGAGGAGGAGGCGGCGGAATCGGCTGCGCCGCATAAGGTCAAGCAC** Boy ine 10 **GCACCCAAGTCGGCCCAEGAAAAGGCGGCGGAATCGACTGCGCCACATAAGGTTAAGCAC** KPM3101 -**GCACCCAAGTCCGCCGTAGAAAAAGCGGCCGAATCGACTGGCCCACACAAGGTTAAGCAC KPM3401** GCGCCGAAGTCGGCGCGCAAGAGGCCGAAGAACGGACCA----CGCAGAAAGTCAAGCAC ATCC51789 T801 GCGCCGAAGTCGCCAAGTGAACGCGCAGCCGAATCCACTGCACCGCACAAAGTTAAGAGC **GCGCCGAAGTCGCAAGTGAACGCCCAGCCGAATCCACTGCACCGCACAAAGTTAAGAGC** T901 T704 GCGCCGAAGTCGCCAAGTGAACGCGCAGCCGAATCCACTGCACCGCACAAAGTTAAGAGC **GCGCCGAAGTCGGCAAGTGAACGCGCAGCCGAATCCACTGCACCGCACAAAGTTAAGAGC** T021 GCCCGAAGTCGGCCAGTGAGAAGGCGGCCGAATTCACCGCCCCCACAAGGTGAAGAAG KPM3504 GCCCCAAAGTCGGCCAGCGAGAAGGCGGCCGAATCCGCCGCCCCGCACAAGGTCAAGAAG 420 KPM1001 * ** ** ** Sequences No. 3, 5 CGCACCTTCCACTATCCCGGTGGTCTGGTCGACTTCGTCAAACACATCAACCGCACCAAA KPM2201 CGCACCTTCCACTACCCCGGCGCTTGGTCGACTTCGTCAAGCACATCAACCGGACCAAG ATCC25274 CGCACGTTCCACTACCCGGGTGGGTTGGTGGATTTCGTCAAGCACATCAATCGCACCAAA **KPM1403** CGCACCTTCCACTACCCCGGCGGTCTGGTCGACTTCGTCAAGCACATCAACCGCACCAAG KPM2027 **KPM1201** CGCACCTTCCACTATCCCGGCGGTTTGGTCGATTTCGTCAAGCACATCAACCGCACCAAG **KPM2403** CGCACTTTCCATTACCCCGGCGGGCTGATCGACTTCGTCAAGCACATCAACCGGACCAAG CGCACCTTCCACTACCCCGGCGGCCTGGTCGACTTCGTCAAACACATCAATCGCACCAAA KPM3012 CECACCTTCCACTACCCCGGCGGCCTGGTCGACTTCGTCAAACACATCAATCGCACCAAA Bov ine 10 KPM3101 CECACCTTCCACTACCCCGGCGGTCTGGTCGACTTCGTCAAGCACATCAACCGCACCAAG KPM3401 CGCACGTTCCACTACCCGGGCGGCTTGGTGGACTTCGTCAAGCACATCAATCGGACCAAG ATCC51789 CGCACGTTCCATTACCCCGGCGGCTTGGTCGATTTCGTCAAACACATCAACCGCACAAAG 1081 CGCACCTTTCACTATCCGGGTGGCCTGGTGGACTTCGTGAAACACATCAACCGCACCAAG T901 CGCACCTTTCACTATCCGGGTGGCCTGGTGGACTTCGTGAAACACATCAACCGCACCAAG T704 CGCACCTTTCACTATCCGGGTGGCCTGGTGGACTTCGTGAAACACATCAACCGCACCAAG T021 CGCACCTTTCACTATCCGGGTGGCCTGGTGGACTTCGTGAAACACATCAACCGCACCAAG KPM3504 CGTACCTTTCACTATCCCGGTGGCTTGGTTGACTTCGTCAAGCACATCAACCGCACCAAG CGTACCTTCCACTATCCCGGGGGTCTGGTTGACTTCGTCAAGCACATCAACCGGACCAAG 480 **KPM1001** ** ** ** ** ** ** ** ** ** ** ** **

Fig. 5	:
KPM2201	AGCCCGATCCAGCAGAGCGTCATCGACTTCGAAGGCAAAGGCACCGGCCACGAGGTCGAA
ATCC25274	AGCCCGATCCAACAGAGCGTCATCGACTTCGAGGGCAAAGGCACCGGCCACGAGGTCGAG
KPM1403	AACCCGATCCAGCAGAGCGTCATCGACTTCGACGGCAAAGGAACCGGGCACGAAGTCGAG
KPM2027	AGCCCGATCCAGCAGAGCGTCATCGATTTCGACGGCAAGGGCACCGGCCACGAGGTCGAG
KPM1201	AGTCCGATTCAGCAGAGCATCGTCGACTTCGAGGGCCAAGGGCTCCGGCCACGAAGTCGAA
KPM2403	AGCCCGATCCAGCAGAGTGTCGTCGCCTTCGACGGCAAGGGTGAAGGGCACGAGGTCGAG
KPM3012	AACCCCATCCACCAGAGCATCATCGATTTCGGTGGGAAGGGCCCCGGCCACGAGGTCGAG
Bovine10	AACCCCATCCACCAGAGCATCATCGATTTCGGTGGGAAGGGCCCCGGCCACGAGGTCGAG
KPM3101	AGCCCGATCCAGCAGAGCATCATCGACTCGACGGCAAAGGTCCCGGCCACGAGGTCGAG
KPM3401	AACCCGATTCACAACAGCATCGTGGATTTCTCCGGCAAGGGACCGGGCCACGAGGTCGAA
ATCC51789	AACCCCATCCATTCGAGCATCGTCGACTTCTCCGGCAAGGGTCCGGCCACBAGGTCGAG
T801	AACGCGATTCATAGCAGCATCGTGGACTTTTCCGGCAAGGGCACCGGGCACGAGGTGGAG
T901 .	AACGCGATTCATAGCAGCATCGTGGACTTTTCCGGCAAGGGCACCGGGCACGAGGTGGAG
T704	AACGCGATTCATAGCAGCATCGTGGACTTTTCCGGCAAGGGCACCGGGCACGAGGTGGAG
T021	AACGCGATTCATAGCAGCATCGTGGACTTTTCCGGCAAGGGCACCGGGCACGAGGTGGAG
KPM3504	AACGCCATCCACAGCAGCATCGTCGACTTCTCCGGAAAGGGGACCGGCCACGAAGTGGAB
KPM1001	AACGCCATCCACAGCAGCATCGTCGACTTCTCCGGTAAGGGACCCGGCCACGAAGTGGAG 540
	* * ** ** ** ** ** ** ** ** ** ** **
KPM2201	ATCSCGATGCAGTGGAACGGCGGCTACTCCGAATCGGTGCACACCTTCGCCAACACCATC
ATCC25274	ATCGCGATGCAGTGGAACGGTGGCTACTCGGAGTCGGTGCACACCTTCGCCAACACGATC
KPM1403	ATCGCGATGCAGTGGAACGGTGGTTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
KPM2027	ATCGCCATGCAGTGGAACGGCGGCTACTCGGAGTCCGTCC
KP#1201	ATCGCGATGCAGTGGAACGGCGGCTACTCGGAGTCGGTGCACACCCTTCGCCAACACCATC
KPM2403	ATCGCGATGCAGTGGAACGGCGGCTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
KPM3012	ATCGCGATGCAGTGGAACGGCGGCTACTCCGAATCGGTGCACACCTTCGCCAACACCATC
Bovine10	ATCGCGATGCAGTGGAACGGCGGCTACTCCGAATCGGTGCACACCTTCGCCAACACCATC
KPM3101	ATCCCGATGCAGTGGAACGCCGCTACTCGGAATCCCTGCCACACCCTTCGCCAACACCATC
KPM3401	ATCGCGATGCAGTGGAATGCCGGCTACTCGGAGTCGGTGCACACCTTCGCCAACACCATC
ATCC51789	ATCGCAATGCAGTGGAACGCCGGCTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
T801	ATCGCGATGCAATGGAACGCCGGGTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
T901	ATCGCGATGCAATGGAACGCCGGGTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
T704	ATCGCGATGCAATGGAACGCCGGGTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
T021	ATCGCGATGCAATGGAACGCCGGGTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
KPM3504	ATCGCGATGCAGTGGAATGCCGGCTATTCGGAGTCGGTGCACACCTTCGCCAACACCATC
KPM1007	ATCGCGATGCAGTGGAATGCCGGCTATTCGGAGTCGGTGCATACCTTCGCCAACACCATC 600
•	***** ***** ***** * ** ** ** ** ** ** *

Fig. 6	
KPM2201	AACACCCACGAGGGCGCCACCCACGAAGAGGGCTTCCGCAGTGCGCTGACCTCGGTGGTC
ATCC25274	AACACCCACGAGGGCGGTACGCACGAAGAAGGGTTCCGCAGTGCGCTGACGTCGGTGGTG
KPM1403	AACACCCATGAGGGCGCACCCACGAGGAGGGCTTCCGCAGCGCGCTGACCTCGGTGGTG
KPM2027	AACACGCACGAGGGCGCCCCCACGAGGGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
KPM1201	AACACCCATGAGGGTGGAACGCACGAAGAGGGCTTCCGCAGTGCGTTGACCTCGGTGGTG
KPM2403	AACACCCACGAGGCCGCACCCACGAAGÁAGGETTCCGCAGCGCACTGACATCGGTGGTG
KPM3012	AACACGCACGAGGGCGCACCCACGAGGAGGGCTTCCGCAGCGCGCTGACCTCCGTGGTC
Bovine10	AACACGCACGAGGGCACCCACGAGGAGGGCTTCCGCAGCGCGCTGACCTCCGTGGTC
KPM3101	AACACCCACGAGGGCGCCACCCACGAAGAGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
KPM3401	AACACCCACGAGGGCGCACCCACGAAGAGGGCTTCCGCAGCGCGTTGACGTCGGTGGTC
ATCC51789	AACACCCACGAGGGCGCACCCACGAAGAAGGGTTCCGCGCGCACTGACGTCCGTGGTG
T801	AACACCCACGAGGGCGCACCCACGAAGAGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
T901	AACACCCACGAGGCCGCCACCCACGAAGAGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
T704	AACACCCACGAGGGCGCACCCACGAAGAGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
T021	AACACCCACGAGGGCGECACCCACGAAGAGGGCTTCCGCAGCGCGCTGACGTCGGTGGTG
KPM3504	AACACCCATGAGGGCGGGACCCATGAAGAAGGGTTCCGCAGCGCGCTCACGTCCGTGGTG
KPM1001	AACACCCACGAGGGTGGGACCCACGAAGAGGGGTTCCGCAGCGCGCTGACCTCGGTGGTG 660
	***** ** ***** ** ** ** ** ** ** ** **
KPM2201	AACAAGTACGCCAAAGACAAGAAGCTGCTCAAGGAGAAGGACCCGAATCTCACCGGTGAC
ATCC25274	AACAAATACGCCAAAGACAAGAAGCTGCTGAAAGACAAGGACCCGAACCTCACCGGTGAC
KPM1403	AACAAGTACGCCAAAGACAAGAAGCTGCTCAAGGACAAGGATCCCAACCTCACCGGCGAC
KPM2027	AACAAGTACGCCAAAGACAAGAAACTGCTGAAGGACAAAGATCCCAACCTCACCGGTGAC
KPM1201	AACAAGTACGCCAAAGACAAGAAGCTGCTCAAGGACAAGGACCCCAACCTCACCGGTGAC
KPM2403	AACAAGTACGCCAAAGACAAGAAGCTGCTCAAGGAGAAGGACGCCAACCTCACCGGCGAC
KPM3012	AACAAGTACGCCAAGGACAAGAAGCTGCTCAAGGACAAGGACCCCAACCTGACCGGCGAC
Boy ine 10	AACAAGTACGCCAAGGACAAGAAGCTGCTCAAGGACAAGGACCCCAACCTGACCGGTGAC
KPM3101	AACAAGTACGCCAAAGACAAGAAGTTGCTGAAAGACAAGGACCCGAACCTCACCGGCGAC
KPM3401	AACAAATACGCCAAGGACCGCAAACTCCTGAAGGACAAAGACCCCAACCTCACCGGCGAC
ATCC51789	AACAAGTACGCCAAGGACCGAAAACTGCTGAAGGACAAGGACCCCAACCTCACCGGCGAC
T801	AACAAGTACGCCAAGGACCGCAAGCTACTGAAGGACAAGGACCCCAACCTCACCGGTGAC
T901	AACAAGTACGCCAAGGACCGCAAGCTACTGAAGGACAAGGACCCCAACCTCACCGGTGAC
T704	AACAAETACGCCAAGGACCGCAAGCTACTBAAGGACAAGGACCCCAACCTCACCGGTGAC
T021	AACAAGTACGCCAAGGACCGCAAGCTACTGAAGGACAAGGACCCCAACCTCACCGGTGAC
KPM3504	AACAAGTACGCCAAGGACCGCAAACTGCTCAAAGACAAGGACCCCAACCTCACCGGCGAC
KPM1001	AACAAGTACGCCAAGGACCGCAAACTGCTCAAGGAAAAGGACCCCAACCTCACCGGCGAC 720

Fig. 7			
KPM2201	GACATCCGGGAGGGGTTGGCCGCGGTGATCTCGGTGAAGGTCGCCGAACCGCAGTTCGAG		
ATCC25274	GACATCCGCGAGGGACTGGCCGCGTGATCTCGGTCAAGGTCGCCGAACCCCAGTTCGAG		
KPM1403	GACATCCGAGAAGGCTGGCCGCGGTGATCTCCGTGAAGGTCGCCGAGCCGCAGTTCGAG		
KPM2027	GACATCCGTGAGGGCTTGGCCGCGGTCATCTCGGTGAAGGTCGCCGAGCCACAGTTCGAA		
KPM1201	GACATCCGCGAGGGGTTGGCCGCGGTCATCTCGGTECGGGTGGCAGAGCCGCAGTTCGAG		
KPM2403	GACATTCGCEAGGGCCTGGCCGCGGTCATCTCGGTGAAAGTTGCCGAACCGCAGTTCGAG		
KPM3012	GACATCCGCGAGGGTTTGGCCGCGGTGATCTCGGTCAAGGTGAGCGAACCGCAGTTCGAG		
Bovine10	BACATCCGCGAGGGTTTGGCCGCGGTGATCTCGGTCAAGGTGAGCGAACCGCAGTTCGAG		
KPM3101	GACATTCGCGAAGGCCTGGCCGCGGTGATCTCGGTCAAGGTCAGCGAACCGCAGTTCGAG		
KPN3401	GACATCCGGGAAGGCCTGGCAGCGGTCATTTCCGTCAAGGTCAGCGAACCGCAATTCGAG		
ATCC51789	GACATTCGTGAGGGCCTGGCGGCCGTCATCTCGGTCAAGGTCAGCGAGCCGCAGTTCGAG		
T801	GATATCCGGGAAGGCCTGGCCGCTGTGATCTCGGTGAAGGTCAGCGAACCGCAGTTCGAG		
T901	GATATCCGGGAAGGCCTGGCCGCTGTGATCTCGGTGAAGGTCAGCGAACCGCAGTTCGAG		
T704 .	GATATCCGGGAAGGCCTGGCCCCTGTGATCTCGGTGAAGGTCAGCGAACCGCAGTTCGAG		
T021	GATATCCGGGAAGGCCTGGCCGCTGTGATCTCGGTGAAGGTCAGCGAACCGCAGTTCGAG		
KPM3504	GACATCCGGGAAGGGTTGGCCGCGGTGATTTCGGTCAAAGTCAGCGAACCGCAGTTCGAG		
KPM1001	GACATCCGGGAAGGGTTGGCCGCGGTGATTTCGGTCAAGGTCAGCGAGCCGCAGTTCGAG 780		
	** ** ** ** ** *** ** ** ** ** ** ** **		
KPN2201	GGTCAGACCAAGACCAAGCTGGGCAACACCGAGGTCAAGTCGTTCGT		
ATCC25274	GGCCAGACAAAGACCAAGCTGGGCAACACCGAGGTCAAGTCGTTCGT		
KPM1403	GGCCAGACTAAGACGAAACTCGGCAACACCGAGGTCAAGTCGTTTGTCCAGAAAGTCTGT		
KPM2027	GGCCAGACCAAGACAAAGCTGGGCAACACCGAGGTGAAGTCGTTCGT		
KPM1201 .	GGTCAGACGAAGACCAAGCTGGGCAACACCGAGGTCAAGTCGTTTGTCCAGAAGGTTTGT		
KPM2403	GGCCAGACCAAGACCAAACTGGGTAACACCGAGGTCAAGTCGTTCGT		
KPN3012	GGCCAGACCAAGACCGAGCCAAACTGGGCAACACCGAGGTGAAGTCGTTCGT		
Bovine10	GGCCAGACCAAGACCAAACTGGGCAACACCGAGGTGAAGTCGTTCGT		
KP#3101	GGTCAGACCAAGACCAAGCTGGGCAACACCGAAGTGAAGTCGTTCGT		
KPM3401	GGCCAGACCAAAACCAAGCTGGGCAACACCGAGGTCAAGTCGTTCGT		
ATCC51789	GGCCAGACCAAAACCAAACTCGGCAACACCGAAGTCAAGTCGTTTGTGCAGAAGGTCTGC		
T801	GGCCAGACCAAGACCAAGTTGGGCAACACCGAGGTCAAATCGTTTGTGCAGAAGGTCTGT		
T901	GGCCAGACCAAGACCAAGTTGGGCAACACCGAGGTCAAATCGTTTGTGCAGAAGGTCTGT		
T704	GGCCAGACCAAGACCAAGTTGGGCAACACCGAGGTCAAATCGTTTGTGCAGAAGGTCTGT		
T021	GGCCAGACCAAGACCAAGTTGGGCAACACCGAGGTCAAATCGTTTGTGCAGAAGGTCTGT		
KPM3504	GGCCAGACCAAGACGAAACTAGGCAACACCGAGGTGAAGTCGTTCGT		

KPM1001

Fig. 8		•
KPM2201	AACGAACAGCTCACCCACTGGTTCGAGGCCAATCCGTCGGAAGCTAAAACCGTTG	TGAAC
ATCC25274	AACGAACAGCT CACCCACTGGTT CGAGGCCAATCCGT CGGAAGCCAAAACCGTTG	TCAAC
KPM1403	AACBAACAACTCACTCACTGGTTCGAGGCGAACCCGTCGGAAGCTAAAACCGTTG	TAAAC
KPM2027	AACGAGCAGCT CACCCACTGGTT CGAGGCCAACCCAT CCGAGGCBAAAACGGT GG	TGAAC .
KPM1201	AACGAGCAGCTCACCCACTGGTTCGAGGCCAATCCTTCGGAAGCCAAAACCATTG	TGAAC
KPM2403	* AACGAACAGCTGACCCACTGGTTCGAGGCCAACCCGTCGGAAGCCAAAACCGTCG	TGAAC
KPM3012 .	AACGAACAGCT CACCCACTGGTT CGAAGCCAACCCCGCAGACGCCAAAGT CATTG	TCAAC
Bovine10	AACGAACAGCTCACCCACTGGTTCGAAGCCAACCCCGCAGACGCCAAAGTCATTG	TCAAC .
KP#3101	AACGAACAGCTCACCCACTGGTTCGAGGCCAACCCCGCGGACGCCAAGGTGGTGG	TCAAC
KPM3401	AACGAACAGCTCACGCACTGGTTCGAAGCCAACCCGGCGGATGCCAAAACTGTTG	TAAAC
ATCC51789	AACGAACAGCTCACCCACTGGTTCGAGGCCAATCCCAGCGACGCCAAGACCGTCG	TCAAC
T801	AACGAACAGCTGACCCACTGGTTTGAAGCCAACCCCACCGACTCGAAAGTCGTTG	TGAAC
T901 ·	AACGAACAGCTGACCCACTGGTTTGAAGCCAACCCCACCGACTCGAAAGTCGTTG	TGAAC
T704	AA <u>TGAACAGCTGACCCACTGG</u> TTTGAAGCCAACCCCACCGAC <u>TCGAAAGTCGTTG</u>	TGAAC
T021	AACGAACAGCTGACCCACTGGTTTGAAGCCAACCCCACCGACGCGAAAGTCGTTG	TGAAC
KPM3504	AATGAACAGCTCACCCATTGGTTCGAGGCCAACCCCGCTGATGCTAAAACCGTTG	TCAAC
KPM1001	AACGAACAGCTCACCCATTGGTTCGAGGCCAACCCCGCTGACGCTAAAACCGTTG	TCAAC 900
	** ** ** ** ** ** ** ** ** ** ** ** **	* ***
	Sequences No. 51, 57 Sequences No. 51, 57	
KPM2201		. 47, 53
KPM2201 ATCC25274	Sequences No. 51, 57 Sequences No.	. 47, 53 . TGCGC
	Sequences No. 51, 57 Sequences No. AAAGCGGTGTCCGCCCAAGGCGCGAAAGCGCGAAAGCGCGAAAGCTGG	. 47, 53 TGCGC TGCGG
ATCC25274 KPM1403 KPM2027	Sequences No. 51, 57 AAAGCGGTGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG	47, 53 TECEC TECEE TECEE
ATCC25274 KPH1403	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGGTAAGGCGCGGGAGTTGG	47, 53 TECEC TECEC TECEC TECEC
ATCC25274 KPM1403 KPM2027	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGTAAGGCGCGGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGTGAACTGG	TGCGG TGCGG TGCGG TGCGG TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGTAAGGCGCGGGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGTGAACTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCCAAGGCGCGAAGGTTGG	47, 53 TGCGG TGCGG TGCGC TGCGC TGCGT
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGTAAGGCGCGGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGTGAACTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCCAAGGCGCGCAAGGTTGG AAGGCGGTCTCGTCGGCACAGGCGCGTATCGCCGCCCGCAAGGCACAGAGTTGG	47, 53 TGCGG TGCGG TGCGC TGCGC TGCGT TGCGT TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCACAGGCCCGGATCGCGCGCGGAAAGCCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAGGCCCGGAACTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGAACTTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTCTCGTCGGCACAGGCGCGCTATCGCCGCCCCCAAGGCACAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAAAGTTGG	47, 53 TGCGG TGCGG TGCGC TGCGT TGCGT TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCACAGGCCCGGATCGCGCGCGCAAAGCGCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGCAAGGCCCGGAGGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCCCAAGGCGCGCTGAACTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCGCAAGGCGCGCAAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCACGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAAGAGTTGG	47, 53 TGCGG TGCGG TGCGC TGCGT TGCGC TGCGC TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGCAAAGCGCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGCAAGGCCCGAGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGTAAACTGG AAGGCGGTATCCTCGGCCAGGCCA	47, 53 TGCGG TGCGG TGCGC TGCGT TGCGC TGCGC TGCGC TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGGAAAGCCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCCGCGAGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGAAACTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAACTGG	TGCGC TGCGC TGCGC TGCGC TGCGT TGCGC TGCGC TGCGC TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGCAAAGCGCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGCAAGGCCCGGAGAGTTGG AAGCCGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAGGCGCGCGAACTTGG AAGGCGGTATCCTCGGCCAGGCACGTCTCGCCGCCAAGGCGCGCAAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCCGCCAAGGCGCGAGAACTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCAATCGCCGCCCCAAGGCGCGAGAACTGG AAAGCGGTTTCGTCGGCCCAGGCCCCGCATTGCCCCCCCC	47, 53 TGCGG TGCGG TGCGC TGCGT TGCGC TGCGC TGCGC TGCGC TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGTTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGCAAAGCGCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGCAAGGCCCGAGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGGAACTGG AAGGCGGTATCCTCGGCGCAGGCCACGTCTCGCCGCGCAAGGCGCGAAGGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGTATCGCCGCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGAATCGCCGCGCAAGGCGCGAGAACTGG AAAGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCGCGCAGAACTGG AAAGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCGCGCAGAATTGG AAAGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCGCGCAGAATTGG AAAGCGGTTCCTCGGCGCAAGCCCCGCATTGCCGCCCGCAAAGCGCGCAGAATTGG AAAGCGGTTCCTCGGCGCAAGCCCCGTATCGCCGCCCGCAAAGCACAGAATTGG	TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGGAAAGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCGCGCGGAAAGCCCGAGAGTTGG AAAGCGGTGTCGTCGGCTCAGGCGCGCATTGCCGCCCGCAAGGCGCGGAACTGG AAGGCGGTATCCTCGGCGCAGGCACGTCTCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGTATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCCCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAACTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATCGCCGCCCCCAAAGCCGCAGAACTGG AAGGCGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCCGCAGAATTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGCCCGCAAAGCCCGAGAATTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGCCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGCCACGTAAGGCACGAGAGTTGG	TGCGC TGCGC TGCGC TGCGC TGCGT TGCGC TGCGC TGCGC TGCGC TGCGC TGCGC TGCGC TGCGC
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGCTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGCAAAGCGCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCGGCGCGCAAAGCCCCGAGAGTTGG AAAGCGGTGTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAGGCCCGGAACTGG AAGGCGGTATCCTCGGCGCAGGCCCGCATTGCCGCCCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGTATCGCCGCGCAAGGCACGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCAAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATCGCCGCGCAAAGCCGCGAGAACTGG AAAGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCCGCAAAGCGCGCAGAATTGG AAAGCGTTTCGTCGGCCCAAGCCCGTATCGCCGCCCCGCAAAGCGCAGAAATTGG AAAGCGTTTCCTCGGCGCAAGCCCGTATCGCGCCCCGTAAGGCACGAGAGTTGG AAGGCTGTTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG	47, 53 TGCGG TGCGG TGCGG TGCGT TGCGC TGCGC TGCGC TGCGC TGCGC TGCGC TGCGG TGCGG
ATCC25274 KPM1403 KPM2027 KPM1201 KPM2403 KPM3012 Bovine10 KPM3101 KPM3401 ATCC51789 T801 T901 T704	Sequences No. 51, 57 AAAGCGGTGTCGTCCGCCCAGGCGCGGATCGCCGCGCAAAGCGCGAGAGTTGG AAGGCGGTTTCGTCCGCCCAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCGCGCGGAAGGCCCGAGAGTTGG AAAGCGGTGTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAGGCGCGGAACTTGG AAAGCGGTTCCTCGGCGCAGGCCCGCATTGCCGCCCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGTATCGCCGCCGCAAGGCACGAGAGTTGG AAGGCGGTTTCATCAGCGCAGGCGCGCATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAGTTGG AAGGCGGTTTCGTCGGCCCAGGCCCGGATCGCCGCGCAAGGCGCGAGAATTGG AAGGCGGTTTCGTCGGCCCAGGCCCGCATTGCCGCCCGCAAAGCCGCGAGAATTGG AAGGCGTTTCGTCGGCGCAAGCCCGTATCGCGCCCGCAAAGCCGCAGAATTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGCCCCGCAAAGCCAGAGATTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG AAGGCTGTGTCCTCGGCGCAAGCCCGTATCGCGGCACGTAAGGCACGAGAGTTGG	TGCGC

Fig. 9 CGCAAGAGCGCAACCGACCTCGGCGGCCTGCCGGGCAAGCTCGCCGACTGCCGTTCGACG KPM2201 ATCC25274 CGCAAGAGCGCGACCGATTTGGGCGGGCTGCCCGGCAAGCTGGCCGACTGCCGTTCCACC CGTAAGAGTGCTACGGATTTGGGTGGGTTGCCGGGCAAGTTGGCTGATTGCCGCTCGACG **KPM1403** CGCAAGAGCGCCACCGACCTCGGCGGTCTGCCCGGGAAGCTGCCGACTGCCGCTCCACC KPM2027 CGCAAGAGCGCAACCGATCTCGGTGGGCTGCCCGGCAAGTTGGCCGACTGCCGCTCGACA KPM1201 CGCAAGAGCGCTACCGATCTCGGTGGGCTGCCCGGCAAGCTGGCCGACTGCCGCTCCACC **KPM2403** KPM3012 CGCAAGAGCGCAACCGACCTGGGCGGGCTGCCCGGCAAGCTCGCCGACTGCCGGTCGACC CGCAAGAGCGCAACCGACCTGGGCGGGCTGCCCGGCAAGCTCGCCGACTGCCGGTCGACC Bovine10 CGCAAGAGCECCACCGATCTGGGCGGGCTGCCCGGCAAGCTCCCCGACTGCCGCTCGACG KPM3101 CECAAGAGCGCCACCGACCTCGGTGGGCTECCGGGTAAGCTCGCAGACTGCCGCTCCACC KPM3401 ATCC51789 T801 CETAAGAGCGCCACCGACATCGGTGGATTGCCCGGCAAGCTGGCCGATTGCCGTTCCACG CGTAAGAGCGCCACCGACATCGGTGGATTGCCCGGCAAGCTGGCCGATTGCCGTTCCACG T901 T704 CETAAGAGCGCCACCEACATCGGTGGATTECCCGGCAAGCTGGCCGATTGCCGTTCCACG CGTAAGAGCGCCACCGACATCGGTGGATTGCCCGGCAAGCTGGCCGATTGCCGTTCCACG T021 CECAAGAGCGCAACCGATCTGGGCGGACTACCGGGCAAGTTGGCCGACTGCCGCTCGACC **KPM3504** KPM1001 CGCAAGAGCGCAACCGATCTGGGCGGACTACCCGGCAAGCTCGCCGACTGCCGCTCGACC 1020 GATCCCCGCAAATCCGAACTGTATGTGGTGGAGGGGGACTCCGCCGGCGGCTCGGCCAAG KPM2201 ATCC25274 GACCCGCGCAAGTCCGAACTGTATGTGGTGGAGGGTGACTCGGCAGGTGGCTCGGCCAAG **KPM1403** GACCCGCGGAAATCGGAACTGTATGTGGT6GAGGGCGATTCGGCCGGCGGCTCGGCCAAG KPM2027 KPM1201 **KPM2403** KPM3012 Bovine10 KPM3101 **KPM3401** ATCC51789 T801 T901 T704 T021

96

Caminaga Na 42

GACCCCCGTAAGTCCGAATTATATGTGGTGGAGGGTGATTCAGCCGGCGGCTCGGCGAAG

GACCCGCGCAAGTCCGAACTGTATGTGGTGGAGGGTGATTCAGCCGGCGGCTCGGCGAAG 1080

KPM3504

KPM1001

Fig. 10 AGCGGTCGGGATTCGATGTTCCAGGCGATTCTTCCGTTGCGCGGCAAGATCATCAACGTC KP#2201 AGCGGCCGTGACTCGATGTTCCAGGCCATCCTGCCGCTGCGCGGCAAGATCATCAACGTC ATCC25274 AGTGGGCGTGATTCGATGTTCCAGGCGATCTTGCCGCTGCGCGGCAAGATCATCAACGTC **KPM1403** AGCGGGCGCGACTCGATGTTCCAGGCGATCCTGCCGCTGCGCGGCAAGATCATCAATGTC KPM2027 AGTGGCCGCGATTCGATGTTCCAGGCGATCCTGCCGCTGCGCGGCAAGATCATCAATGTC KPM1201 AGCGGCCGCGACTCGATGTTTCAGGCGATACTTCCGTTGCGCGGCAAGATCATCAACGTC KPM2403 AGCGGCCGGGACTCGATGTTCCAGGCCATCCTTCCGCTGCGCGGCAAGATCATCAACGTC KPM3012 AGCGGCCGGGACTCGATGTTCCAGGCCATCCTTCCGCTGCGGCGAAGATCATCAACGTC Boyine 10 AGCGGCCGCGACTCGATGTTCCAGGCCATCCTGCCGCTGCGCGGCAAGATCATCAACGTC KPM3101 AGCGCCGCGACTCGATGTTCCAGGCGATCCTCCCGCTGCGTGGCAAGATCATCAACGTC KPM3401 AGCGGCCGCGACTCGATGTTTCAGGCGATCCTGCCGTTGCGGGGCAAGATCATCAACGTG ATCC51789 AGCGGTCGCGATTCGATGTTCCAGGCGATACTTCCGCTGCGCGGCAAGATCATCAATGTG T801 AGCEGTCGCGATTCGATGTTCCAGGCGATACTTCCGCTGCGCGGCAAGATCATCAATGTG T901 AGCGGTCGCGATTCGATGTTCCAGGCGATACTTCCGCTGCGCGGCAAGATCATCAATGTG **T704** AGCGGTCGCGATTCGATGTTCCAGGCGATACTTCCGCTGCGCGGCAAGATCATCAATGTG T021 AGCEGCCECGACTCGATGTTTCAAGCGATCTTGCCGTTGCGCEBCAAGATCATCAACGTC KPM3504 AGCGGTCGCGACTCGATGTTCCAGGCCATCTTGCCGTTGCGCGGCAAGATCATCAACGTC 1140 KPM1001 KPM2201 GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAAGTCCAGGCCATCATCACCGCG GAGAAGGCCCGCATCGACCGGGTCCTGAAGAACACCGAAGTCCAGGCGATCATCACCGCG ATCC25274 GAAAAGGCCCGCATCGATCGGGTGCTGAAAAACACCGAAGTCCAGGCCATCATCACCGCG **KPM1403** GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAAGTTCAGGCGATCATCACCGCG KPM2027 KPM1201 GAAAAGGCACGCATCGACCGAGTCCTGAAAAACACTGAAGTCCAGGCGATCATCACCGCG KPM2403 GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAAGTCCAGGCGATCATCACCGCG GAAAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAGGTGCAGGCGATCATCACCGCG KPM3012 GAAAAGECCCGCATCGACCGGGTGCTGAAGAACACCGAGGTGCAGGCGATCATCACCGCG Bovine 10 GAGAAGGCCCGCATCGACCGGGTGTTGAAGAACACCGAGGTGCAGGCCATCATCACCGCC KPM3101 GAGAAGGCGCGCATCGACCGGGTGCTGAAGAACACCGAAGTTCAGGCGATCATCACCGCG KP#3401 ATCC51789 GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACTGAGGTGCAGGCGATCATCACCGCG **EAGAAAGCECECATCGACCGGGTGCTAAAGAACACCGAAGTTCAGGCGATCATCACGGCG** T801 GAGAAAGCGCGCATCGACCGGGTGCTAAAGAACACCGAAGTTCAGGCGATCATCACGGCG T901 T704 **GAGAAAGCGCGCATCGACCGGGTGCTAAAGAACACCGAAGTTCAGGCGATCATCACGGCG** GAGAAAGCGCGCATCGACCGGGTGCTAAAGAACACCGAAGTTCAGGCGATCATCACGGCG T021 **KPM3504** GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAAGTCCAGGCGATCATCACCGCG KPM1001 GAGAAGGCCCGCATCGACCGGGTGCTGAAGAACACCGAAGTCCAGGCGATCATCACCGCG 1 2 0 0

Fig. 11	•			
KPM2201	CTGGGCACCGGGATCCACGACGAGTTCGACATCACCAAACTGCGCTACCACAAGATCGTA			
ATCC25274	CTGGGTACCGGTATTCACGACGAGTTCGACATTTCTAAACTGCGTTACCACAAGATCGTG			
KPM1403	CTGGGCACCGGCATCCACGACGAATTCG/	ACATCACCAAACTGCET	TACCACAAGATCGTG	
KPM2027	CTGGGTACCGGGATTCACGACGAGTTCGACATCACCAAGCTGCGCTATCACAAGATCGTG			
KPM1201	TTGGGTACCGGTATTCACGACGAATTCGACCTCTCGAAGCTGCGCTATCACAAGATCGTC			
KPM2403	CTEGGTACCGGAATTCACGACGAGTTCGACCTCGCCAAACTGCGCTACCACAAGATCGTG			
KPM3012	CTEGGCACCEGGATTCACGACGAGTTCGACATCACCAAGCTECECTACCACAAGATCGTG			
Bovine10	· CTGGGCACCGGGATTCACGACGAGTTCGACATCACCAAGCTGCGCTACCACAAGATCGTG			
KP#3101	CTGGGCACCGGCATCCACGACGAGTTCGACATCACCAAGCTGCGCTATCACAAGATCGTG			
KPM3401	CTGGGCACGGGGATTCACGACGAGTTCGACATCACCAAGCTCCGGTACCACAAGATCGTG			
ATCC51789	CTGGGCACCGGGATTCACGACGAGTTCGACATCTCCAAGCTBCGCTACCACAAGATCGTG			
T801	CTGGGCACCGGGATCCACGACGAGTTCGATATCGGCAAGCTGCGCTACCACAAGATCGTG			
T901	CTGGGCACCGGGATCCACGACGAGTTCGATATCGGCAAGCTGCGCTACCACAAGATCGTG			
T704	CTGGGCACCGGGATCCACGACGAGTTCGA	ATATOGGCAAGCTGCGC	TACCACAAGATCGTG	
T021	CTGGGCACCGGGATCCACGACGAGTTCGATATCGGCAAGCTGCGCTACCACAAGATCGTG			
KPM3504	TTGGGCACCGGTATTCACGACGAATTCGACATCGCGAGACTGCGTTACCACAAGATCGTG			
KPM1001	TTGGGTACCGGCATCCACGACGAATTCG/	ACATCGCGAGACTGCGT	TACCACAAGATCGTG 1260	
		* * * **		
KPM2201	TTG	KPM1403	M. simiae	
ATCC25274	TTG	. KPM1201	M. marinum	
KPM1403	TTG	KPM2201	M. gordonae	
KPM2027	CTG	ATCC25274	M. asiaticum	
KPM1201	TTG .	KPM2027	M. scrofulaceum	
KPM2403	CTG	KPM2403	M. szulgai	
KPM3012	TTG	KPM3012	M. avium	
Bovine10	TTG	Bovine10	M. paratuberculo	
KPM3101	CTG	KPM3101	M. intracellular	
KPM3401	CTG	KPM3401	M. malmoense	
ATCC51789	CTG	ATCC51789	M. branderi	
T801	CTG -	-T801	M. africanum	
T901	CTG	T901	M. microti	
T704	ста	T704	M. bovis	
T021	ств	T021	M. tuberculosis	
KPM3504	CTG	KPM3504	M. gastri	
KPM1001	СТС	KP#1001	M. kansasii	

Fig. 12

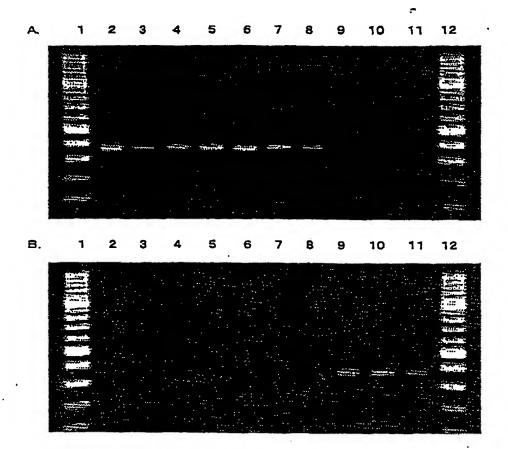


Fig. 13

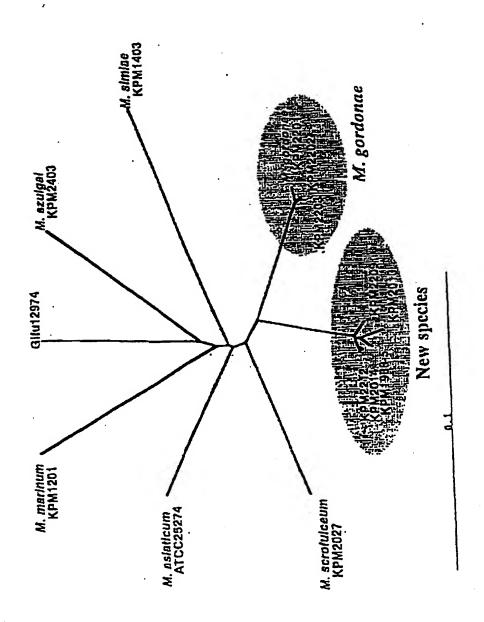


Fig. 14

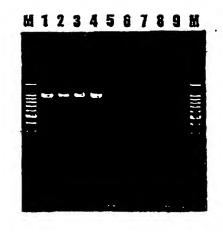


Fig. 15

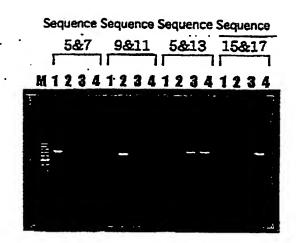
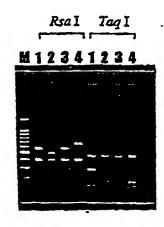


Fig. 16



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